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Possible Long-Term Health Effects of Short-Term Exposure to Chemical Agents

Volume I
Anticholinesterases and Anticholinergics

POSSIBLE LONG-TERM HEALTH EFFECTS OF SHORT-TERM EXPOSURE TO CHEMICAL AGENTS

Volume 1
Anticholinesterases and Anticholinergics

prepared by the

Panel on Anticholinesterase Chemicals
Panel on Anticholinergic Chemicals

Committee on Toxicology
Board on Toxicology and Environmental Health Hazards
Assembly of Life Sciences

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PREFACE

The Department of the Army asked the Committee on Toxicology (COT) of the National Research Council (NRC) Assembly of Life Sciences to conduct a study of the possible chronic adverse health effects on servicemen of experimental exposure to various chemicals at U.S. Army Laboratories at Edgewood, Maryland, in about 1958-1975. The Edgewood tests were conducted to learn how specific chemicals may affect humans. Some 6,720 soldiers took part in the program; 254 chemicals were administered by various routes. Chemicals were tested for various military applications. Among them were acutely toxic anticholinesterase chemicals; incapacitating agents, which included the glycolates, atropine-like anticholinergic compounds of which BZ (3-quinuclidinyl benzilate) is a prototype; the indoles, represented by EA 1729 (LSD-25); the cannabinols, or marijuana-like compounds; and the sedative, or "tranquilizer," group.

Review of the Edgewood tests included interviews with key administrators, investigators, nurses, and technicians. The account of practices and procedures undertaken at Edgewood comes from a wide spectrum of sources, both documents and interviews. They represent a wide spectrum of attitudes, but are essentially in agreement. Committees were formed at Edgewood to review classified chemicals and reports for declassification and use by NRC panels. Extensive extracts were prepared of preclinical animal and human protocols and technical reports at Edgewood Libraries and other Edgewood facilities where records of subjects and details of exposure conditions and clinical findings are maintained. A repository was established at Edgewood Arsenal (August 1980) for storing the reports and information obtained from other sources.

The NRC staff organized the test chemicals into several pharmacologic classes; the first two classes consisted of 15 anticholinesterase (Appendix A) and 24 anticholinergic (Appendix B) chemicals. Two expert panels were established (on anticholinesterases and on anticholinergics) with chairmen selected from the COT and members drawn from the scientific community on the basis of familiarity with some aspect of the pharmacologic class involved or expertise in a discipline needed for proper evaluation of potential adverse health effects.

Research and experimental case files on volunteers were extracted and summarized. Digests of the literature were prepared.

The charge of the two panels was to determine whether, on the basis of the data available, it is possible to demonstrate the likelihood of long-term health effects or delayed sequelae and, if so, whether the involved chemicals, as tested, are likely to produce long-term health effects or delayed sequelae. The charge did not include policy recommendations regarding human testing. This report is the first of two on the Edgewood tests. It presents the two panels' tentative conclusions which are based on a review of data obtained from Edgewood in nine documents provided by NRC staff (listed in Appendixes C and D), on mortality data organized by the NRC Medical Follow-Up Agency, and on separate discussions and papers generated by the panel members. Specific issues addressed were based on this information and relevant items from the published literature.

A second report will contain an evaluation of most of the remaining chemicals tested at Edgewood and an evaluation of the effects on morbidity state of the test subjects. The information on morbidity may permit firmer conclusions than are presented here.

EXECUTIVE SUMMARY

In response to a request from the Department of the Army, the Committee on Toxicology (COT) of the National Research Council Assembly of Life Sciences conducted a study to evaluate the possibility of long-term or delayed adverse health effects of chemical agents tested on military volunteers during the 1960s and 1970s.

The task was begun about 2 years ago, with interviews of key people who had been associated with the soldier-volunteer test program of the Army Chemical Center (Edgewood Arsenal), Maryland. Initial efforts included a thorough review of the Army's laboratory and clinical records and of reports in the scientific literature. Some 6,720 soldiers took part in the Edgewood program as test subjects in about 1958-1975, and 254 chemicals were administered in an experimental setting.

The chemicals were divided into eight major pharmacologic classes and organized within each class according to structure. The most extensively studied classes are the anticholinergic and the anticholinesterase chemicals, and these are the subjects of this report; the other classes will be reported on later. Panels were then formed to study these two main classes. The chairmen were selected from the COT, and the members were selected for expertise in some aspect of the review of the pharmacologic class in question.

The anticholinesterases are generally organophosphates; these are nerve agents resembling parathion. Major symptoms of low-level anticholinesterase exposure include salivation, increased sweating, contracted pupils, and bronchospasm. The anticholinergics are generally "glycolates", (substituted glycolic and tropic acid esters) of which the representative and best-known member is atropine. Major symptoms of low-level atropinization include dry mouth, dilated pupils, and tachycardia. There were 24 anticholinergics tested on about 1,800 subjects. There were 15 anticholinesterases tested on about 1,400 subjects. These two classes are readily paired, in that members of each are used as treatment for overexposure to members of the other.

The next step involved organization of Edgewood data and reports. Some of this material had to be declassified before use by the panels. Digests of the entire available literature, classified and unclassified, were prepared by consultant pharmacologists, and various documents were made available to the panels for their use in investigating the possibility that the Edgewood test experience resulted in persistent adverse effects. The panels met several times, beginning in June 1980.

The specific charge to the two panels was to determine:

o Whether the data available are sufficient to estimate the likelihood that the test chemicals have long-term health effects or delayed sequelae.

o Whether the involved chemicals, as tested, are likely to produce long-term adverse health effects or delayed sequelae in the test subjects.

ANTICHOLINESTERASE CHEMICALS

The panel concludes that although no evidence has been developed (to date) that any of the anticholinesterase test compounds surveyed carries long-range adverse human health effects in the doses used, the results of an ongoing NAS/NRC morbidity study may shed further light on this issue. The panel therefore is unable to rule out the possibility that some anti-ChE agents produced long-term adverse health effects in some individuals. Exposures to low doses of OP compounds have been reported (but not confirmed) to produce subtle changes in EEG, sleep pattern, and behavior that persist for at least a year. Whether the subjects at Edgewood incurred these changes and to what extent they might now show these effects are not known. If such changes occurred and persisted, they would be difficult to detect now. They could be determined scientifically only by a new study in which EEG, sleep state, and psychologic-test scores were compared with those from nonexposed control subjects. This might be considered, if reasonable suspicion develops, based on responses obtained in the referenced morbidity study, that selected subjects experienced behavioral changes traceable in onset to experimental exposure to the anti-ChE agents.

ANTICHOLINERGIC CHEMICALS

No firm evidence has been seen that any of the anticholinergic test compounds surveyed produced long-range adverse human health effects in the doses used at Edgewood Arsenal. More intensive study is required to confirm this conclusion. The high frequency of uncontrolled variables makes evaluation of behavioral effects difficult.

On the basis of available data, in the judgment of the panel, it is unlikely that administration of these anticholinergic compounds will have long-term toxicity effects or delayed sequellae. An ongoing morbidity study should provide more definitive information once it is completed.

MORTALITY

Standardized mortality ratios were derived from mortality data for the soldiers (all males) who participated in the Edgewood Tests and from U.S. mortality rates. For each class of chemicals, the mortality rates among the soldiers were not significantly higher than the rates of the U.S. population, categorized by age and calendar year.

MORBIDITY

An ongoing morbidity study among the test subjects is expected to provide a more complete understanding of the long-term consequences of exposure to anticholinergic and anticholinesterase chemicals.

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INTRODUCTION

HISTORY OF THE EDGEWOOD TESTING PROGRAM

Human experimentation appears to have been an integral part of the history of the U.S. Army chemical warfare (CW) research efforts until its suspension in 1975. On June 28, 1918, the President directed the establishment of the Chemical Warfare Service (CWS). Four years later, in October 1922, the CWS created a Medical Research Division to conduct research directed at providing a defense against chemical agents. No matter how exhaustively an agent was tested in animals, it was felt that its efficacy in humans also had to be studied.

In early 1941, the threat of war increased the urgency of the development of protection against CW agents and, consequently, engendered a need for a larger source of volunteers. Formal authority to recruit and use volunteer subjects in CW experiments was initiated in 1942. The Secretary of War was asked to rule on the permissibility of using enlisted men for testing agents of the mustard-gas type. In July 1943, the CWS was assigned responsibility for all medical research related to CW. This extension of the CWS mission included toxicologic research and the study of hazards to the health of personnel in the CWS.

The issue of the use of human volunteers was considered by the Armed Forces Medical Policy Council during the early 1950's. The Council concluded that essential data could not be obtained unless human volunteers were used, and the use of humans in medical research was authorized. By 1954, the Chemical Corps (formerly CWS) had established a framework within which to conduct human experimentation, but it lacked an adequate pool of volunteers. In 1955, it was decided that the most practical source of volunteers would be enlisted men stationed at Army installations in the vicinity of Edgewood Arsenal. It was emphasized that voluntary consent of each human subject was absolutely essential. It was also stated that, in all experiments involving volunteer subjects, the subjects would be thoroughly informed of all procedures and of what might be expected as a result of each test. Furthermore, each volunteer would be free to determine whether he desired to participate in a given experiment. In October 1959, approval was granted for the conduct of research on volunteers to investigate defense against incapacitating CW agents.

The search for incapacitating agents intensified when the Kennedy administration took office. The involvement with incapacitating agents represented a departure from an earlier period, begun in 1946, when interest in highly toxic (acute) anticholinesterase chemicals resulted from their development in Germany during World War II. The basic purpose of a military incapacitating agent is to produce temporary ineffectiveness without permanent injury or death. Incapacitating agents (anticholinergic chemicals) and highly toxic (acute) anticholinesterase chemicals produce functional and structural effects on the nervous system which cause rapid or delayed effects on an individual's performance and behavior.

PROCEDURES USED AT THE EDGEWOOD CHEMICAL TESTING PROGARM

A fairly extensive discussion of the procedures used is provided in the Inspector General's report, Use of Volunteers in chemical Agent Research, prepared by Colenel James R. Taylor and Major William H. Johnson and dated March 1976 (listed in Appendixes C,D).

RECRUITMENT OF VOLUNTEERS

Recruiting teams (initially administrative officers, but later often including military physicians from the Edgewood laboratory) visited Army installations where a briefing, usually with a film and handouts, was presented to a large number of enlisted men. Generally 10 to 20 percent of the audience expressed interest and these men were asked to complete a personal history, which included medical and psychologic items and the Minnesota Multiphasic Personality Inventory (MMPI). It was not unusual for 400-600 men to request assignment in the course of a tour of seven to ten installations. Of these, no more than 100 were selected and eventually assigned for a 1- to 2- month period of temporary duty at Edgewood Arsenal.

The "incentives" for volunteering consisted of a small monetary allowance (approximately $1.50 a day for temporary duty), the assignment of only light duties while at Edgewood, and almost every weekend free. Some volunteers were genuinely interested in the scientific and experimental aspects; however, if curiosity or the desire to "test one's self" seemed too strong, the applicant was usually not accepted.

As a group, the volunteers were above average in physical and mental qualifications, with a mean IQ near 110, good behavior records, and "normal" MMIPs with profiles generally within two standard deviations of the population mean on all scales.

GUIDELINES FOLLOWED IN THE PROTECTION OF SUBJECTS

The Nuremberg and Helsinki guidelines were regarded by the investigators and their supervisors as appropriate constraints in studies performed on volunteers, although this was not clearly articulated in official memoranda until the mid-1960s. The provision of accurate, informative explanations of what was planned and what might be expected was regarded as essential to the continuance of the program. Written consents, witnessed by medical staff members, were required from the outset and became more elaborate with time. However, minutes of hearings conducted by the U.S. Senate Subcommittee on Health and Subcommittee on Administrative Practice and Procedure, September 10-12, 1975, stated that the consent information was inadequate by current standards.

INVESTIGATORS

When BZ studies were begun in 1960, the need for a psychiatrist with biologic training and interest was recognized, and one was assigned to the program in January 1961. Physicians trained in internal medicine, anesthesiology, cardiology, surgery, dermatology, ophthalmology, neurology, and other specialties were assigned as the program proceeded. Many were research-oriented and have since gained excellent reputations in academic medicine at leading universities.

SELECTION OF DOSES FOR HUMAN TESTS

Subthreshold doses based on estimates from animal potency studies were used in the first few subjects. For example, the earliest exposures to BZ, one of the anticholinergic test compounds, were at doses between 0.1 and 0.5 µg/kg, which was less than one-tenth the incapacitating dose (ID) ultimately established at approximately 5.5. µg/kg. The intravenous route was preferred initially, but other routes of administration were also used. Inhalation studies were sometimes undertaken after a compound had been thoroughly studied by one of these parenteral routes. Oral and percutaneous studies were performed when effectiveness via these routes was of interest.

As the program developed, it became customary to test agents at dose increments of 40 percent, once the approximate effects of the lower doses were known. Placebos were used in some studies, but the cost with respect to subject confinement time, staff workload, and delay in achieving estimates of potency made this impractical except in special cases (e.g., evaluation of antagonists). Instead, low and high doses were assigned in a randomized manner by someone not involved in an experiment. Placebo responses were minimal. Signs of drug effects at all but the lowest doses were significant and made the value of placebo or "no treatment" inconsequential.

RANGE OF DOSES

Rarely did the intramuscular or intravenous doses exceed 1.5 times the incapacitating dose. Inhalation doses were higher, but potencies were lower by this route (usually about 60 percent of that by the intravenous or intramuscular route). Compared with doses described in the scientific literature on atropine coma therapy (18-23) or scopolamine therapy (19), the BZ doses to which volunteers were exposed appear modest. As much as 20 times the ID_{50} of atropine and 30-40 times the ID_{50} of scopolamine have been administered in the past by clinicians--often to older and less robust patients. Many patients received multiple exposures of this magnitude over a period of days or weeks. these therapeutic procedures, reported several decades ago in refereed journals, actually stressed and advocated the benefits of such treatment, despite occasional deaths (most of which appear to have been caused by hyperthermia).

SAFETY MARGIN

The safety margin of a drug is defined as the ratio of the lethal dose (LD) to the effective dose (ED). Sometimes, ratio of the LD_{50} to ED_{50} is used, although a more conservative approach favors the use of the ratio of LD_1 to ED_{99} (standard margin of safety). In the case of incapacitating agents, much reliance is placed on extrapolation from animal experimentation, and estimation of the LD_1 is generally unreliable.

Many other extrapolation techniques have been used in manipulation of animal lethality data in an effort to generate a reasonable human estimate. By taking a conservative approach with data on deaths at low doses, one can derive estimates for man that are modest and in keeping with clinical judgement. Such methods depend on procedures developed and applied in toxicology.

ANTICHOLINESTERASES

Anticholinesterases (anti-ChEs) are toxic to humans principally because they interfere with molecular and cellular mechanisms required for the normal functioning of the central nervous system (CNS) and peripheral nervous system (PNS). Their adverse health effects are related mostly to inhibition of acetylcholinesterase (AChE), a critically important CNS and PNS enzyme that hydrolyzes the neurotransmitter acetylcholine (ACh). Chemical-warfare (CW) agents exploit the acute, life-threatening properties of profound AChE inhibition; some of the anti-ChEs precipitate other clinically significant deleterious effects on sensory and neuromuscular function. All the major anti-ChEs are chemically reactive and potentially capable of alkylating a variety of biologic macromolecules, but the long-term health implications of these reactions are not well known. This chapter therefore focuses on the acute toxic effects of anti-ChEs and the possible long-term effects on CNS and PNS function.

The fundamental cellular component of the nervous system, the neuron, has long, branching, cylindric processes (dendrites and axon) that extend from the cell body. Dendrites are modified for signal reception and transduction and form extensive networks that permit interneuronal communication, coordination, and integration of nervous-system function. The axon typically is a long extension of the neuron specialized for transmission of electric signals and, at its distal end, for chemical communication of information to other neurons or to muscle at sites termed synapses and neuromuscular junctions, respectively.

ACh is the chemical transmitter of information from both somatic and autonomic (PNS) neurons. On the somatic side, the lower motor neuron uses ACh to convey excitatory impulses to voluntary muscle. Cholinergic neurons of the autonomic division of the PNS are grouped in craniosacral (parasympathetic) and thoracolumbar (sympathetic) outflows from the spinal cord. Parasympathetic pathways use ACh at both preganglionic and postganglionic neurons; in the sympathetic system, ACh is restricted to the preganglionic effector. These cholinergic-dependent autonomic neurons play an important role in regulating the function of various vitally important effector organs. Cholinergic pathways are widely distributed in CNS tissue, but their functions are less well understood than those in the PNS.

AChE terminates the transmitter action of ACh. Drugs that inhibit or inactivate AChE (anti-ChE agents) cause ACh to accumulate at cholinoceptive sites and thus produce effects equivalent to continuous stimulation of cholinergic nerve fibers. Before World War II, only "reversible" anti-ChE agents were generally known, of which physostigmine (eserine) is the outstanding example. Shortly before and during World War II, a class of highly toxic chemicals, the organophosphorus compounds (OPs), were developed, chiefly by Schrader of I.G. Farbenindustrie,first as agricultural insecticides and later as CW agents. The high potency of these compounds was found to be due to "irreversible" inhibition of AChE; thus, they produced effects for considerably longer periods than the classical inhibitors. Since the pharmacologic actions of both classes of anti-ChE agents are qualitatively similar, they are discussed as a group, and important features of individual classes or compounds are noted.

CHEMISTRY OF ANTICHOLINESTERASES

A complete list of anti-ChE compounds used in the Edgewood program is contained in the master file (Appendix A). Structure-activity relationships have been reviewed extensively for the "reversible" inhibitors (1,2) the OP agents (3,4) and both classes of compounds (5-7).

"REVERSIBLE" INHIBITORS

After the structure of physostigmine was established, Stedman (8,9) undertook a systematic investigation of a number of related synthetic compounds. The essential moiety of the physostigmine molecule was found to be the methylcarbamate of a basically substituted, simple o-aminophenol. The quaternary ammonium derivative, neostigmine, is a compound of greater stability and equal or greater potency. Retention of the dimethylcarbamate side chain in the meta position, but with incorporation of the quaternary nitrogen atom into the ring to form a pyridyl nucleus, results in compounds with anti-ChE and other pharmacologic properties similar to those of neostigmine. Pyridostigmine is a drug of this class.

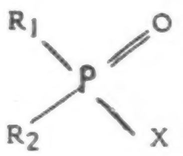
Although the carbamates are the most familiar and the most commonly encountered anti-ChEs, members of other chemical classes are also capable of inhibiting AChE. For example, edrophonium, an analogue of the phenolic residue of neostigmine, is used extensively in clinical medicine. It was not administered to the volunteers at Edgewood, but two other noncarbamates were: 1,2,3,4-tetrahydro-9-acridinamine (Tacrine) and hexafluorenium (Mylaxen). These compounds also are used in clinical medicine but are not as popular as neostigmine, pyridostigmine, or edrophonium.

ORGANOPHOSPHORUS (OP) INHIBITORS

The general formula for this class of cholinesterase inhibitors is shown in Figure 1. A great variety of substituents is possible: R_1 and R_2 may be alkyl, alkoxy, aryloxy, amido, mercapto, or other groups; and X (also called the "leaving group") may be a halide, cyanide, thiocyanate, phenoxy, thiophenoxy, phosphate, alkylthioethylmercaptide, dialkylaminoethylmercaptide, or carboxylate group. It is obviously impossible to discuss here more than a few representative compounds of the more than 50,000 that

Fig. 1: Organophosphorus Compounds

General Formula:



have been prepared; a useful chemical classification of the compounds in this class that are of particular pharmacologic or toxicologic interest has been developed by Holmstedt (3,4). Diisopropyl phosphorofluoridate (DFP) is perhaps the best known and the most extensively studied compound.

Generally, the most acutely toxic compounds are those with one carbon-phosphorus bond (phosphonates). The compounds studied by the Army (Appendix A), with the exception of DFP, contain this feature. In GA (tabun), the leaving group is cyanide. In GB (sarin), GD (soman), and GF, the leaving group is fluoride. In the V agents, VX and EA 3148 (the most potent agent administered to the volunteers), the leaving group is a dialkylaminoalkylmercaptide.

ABSORPTION, FATE, AND EXCRETION OF ANTI-CHES

Physostigmine is readily absorbed from the gastrointestinal tract, subcutaneous tissues, and mucous membranes. It undergoes hydrolytic cleavage at the ester linkage by cholinesterases; renal excretion plays only a minor role in its disposal. In man, a 1-mg dose of physostigmine injected subcutaneously is largely destroyed in 2 h. Neostigmine and related quaternary ammonium drugs are absorbed poorly after oral administration, and much larger doses are needed for effect than when they are administered by injection; both are metabolized by hepatic microsomal enzymes (10) and excreted in the urine.

The commonly encountered OP anti-ChE agents are, with some exceptions (e.g., echothiophate), highly soluble in lipids. Consequently, they are rapidly and effectively absorbed when administered by almost any route, including the gastrointestinal tract, the skin and mucous membranes after contact with the liquid form, and the lungs after inhalation of vapors, finely dispersed dust, or aerosols. Most OP compounds are excreted almost entirely as metabolites in urine. Between the time of absorption and excretion, there are varied periods during which the original compound or its metabolites remain bound to proteins in the blood and tissues.

Both hydrolytic and oxidative enzymes are involved in metabolism of the OP compounds. The OP anti-ChE agents are hydrolyzed in the body by a group of enzymes, the phosphorylphosphatases. These are widely distributed and hydrolyze a large number of OP compounds (e.g., DFP, tabun, sarin, paraoxon, and tetraethyl pyrophosphate, or TEPP) by splitting the anhydride-like P-F (or P-CN) bond. They also hydrolyze several aliphatic esters (e.g., ethyl acetate) and aromatic esters (e.g., phenyl acetate). The enzymes are not irreversibly inhibited by OP compounds, presumably because the phosphorylated active site reacts rapidly with water to regenerate the free form, in contrast with its high stability in the case of the cholinesterases.

Because of the above reactions, the effects of exposure to two OP insecticides may be synergistic. For example, when malathion is administered to animals in combination with O-ethyl O-p-nitrophenyl phenylphosphonothionate (EPN), the resulting toxicity is as much as 50 times that expected from the sum of their individual toxicities, this results primarily from the inhibition by EPN of enzyme systems that normally metabolize malathion to inactive products. Other combinations of OP insecticides also have shown supra-addition of toxic effects.

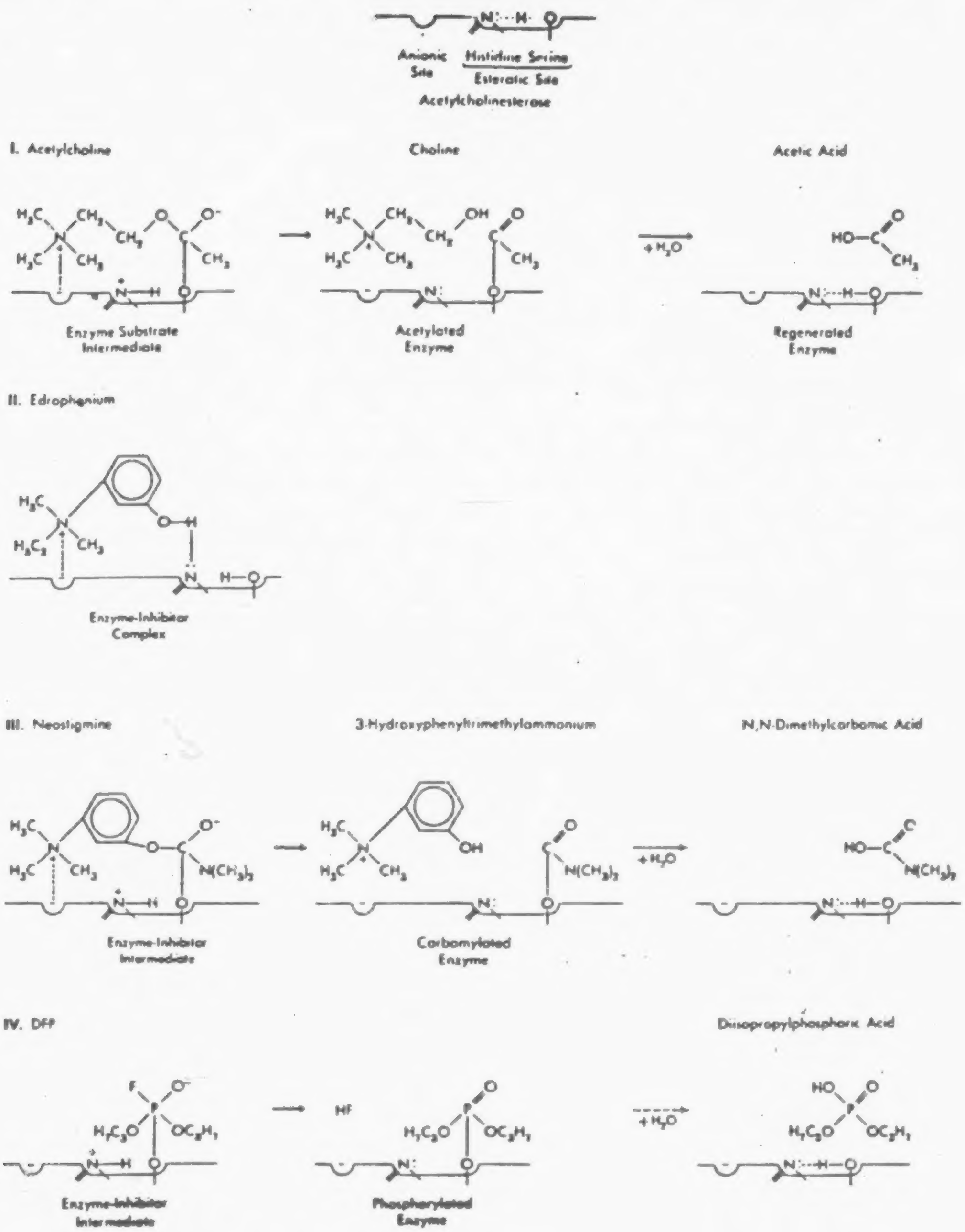

Anionic Site
Histidine Serine
Esteratic Site
Acetylcholinesterase
I. Acetylcholine
Choline
Acetic Acid
+H₂O
Enzyme Substrate Intermediate
Acetylated Enzyme
Regenerated Enzyme
II. Edrophonium
Enzyme-Inhibitor Complex
III. Neostigmine
3-Hydroxyphenyltrimethylammonium
N,N-Dimethylcarbamic Acid
+H₂O
Enzyme-Inhibitor Intermediate
Carbamylated Enzyme
IV. DFP
HF
Diisopropylphosphoric Acid
+H₂O
Enzyme-Inhibitor Intermediate
Phosphorylated Enzyme

Fig. 2: Steps involved in the hydrolysis of acetylcholine (ACh) by acetylcholinesterase (AChE) (I), and in the inhibition of AChE by reversible (II), carbamyl ester (III), and organophosphorus (IV) agents. Heavy, light, and dashed arrows represent extremely rapid, intermediate, and extremely slow or insignificant reactions, respectively. Reproduced from Koelle, G.B., In:The Pharmacologic Basis of Therapeutics (Goodman, L.S. and Gilman, A., eds.), 5th ed. Macmillan Publ. Co., 1975, pg. 448.

MECHANISMS OF ACTION

INHIBITION OF ACETYLCHOLINESTERASE BY ANTICHOLINESTERASES

The mechanisms of action of compounds that typify the three classes of anti-ChE agents are shown in Figure 2. They differ primarily in quantitative respects from the reaction between AChE and its normal substrate, ACh.

Simple quaternary compounds, such as edrophonium, form electrostatic bonds with the anionic site of the enzyme and hydrogen bonds with the imidazole nitrogen atom of the esteratic site. In all such cases, inhibition is rapidly reversible, and such drugs have a very short duration of inhibitory action.

It was at one time generally assumed that physostigmine, neostigmine, and related inhibitors that possess a carbamyl ester linkage or urethane structure, in addition to a tertiary amino or quaternary ammonium group, inhibit the enzyme in the same reversible fashion. However, careful kinetic studies showed that physostigmine and neostigmine are hydrolyzed by cholinesterase (11-14). Initially, inhibitors of this class form complexes in which the inhibitor is attached to the enzyme at both anionic and esteratic sites; subsequently, hydrolysis proceeds in a manner analogous to that of ACh, and the alcoholic moiety is split off, leaving a carbamylated and inhibited enzyme. Later, this enzyme reacts with water to release a substituted carbamic acid and the regenerated enzyme. The main difference between the reaction of the natural substrate, ACh, and that of the carbamate inhibitors is the velocity of the final step; the half-life of dimethylcarbamyl AChE, formed by the reaction with neostigmine, is more than 40 million times that of the acetylated enzyme: 30 min and 42 μs, respectively (15).

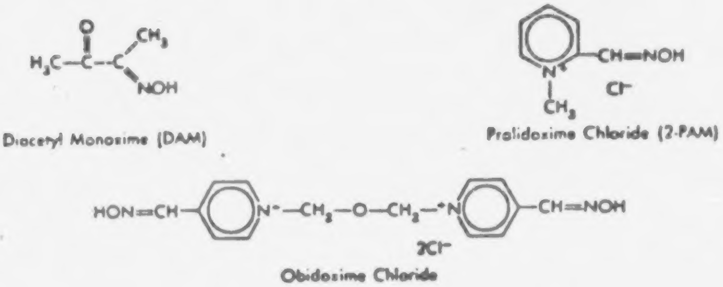
The reaction between AChE and most OP inhibitors, such as DFP, occurs only at the esteratic site. It proceeds in a comparable fashion, except that, as a result of the initial hydrolysis, the enzyme becomes phosphorylated. The resulting phosphorylated enzyme is extremely stable: if the attached alkyl groups are methyl or ethyl, substantial regeneration of the enzyme by hydrolytic cleavage requires several hours; with isopropyl groups, as in DFP, virtually no hydrolysis occurs, and the return of AChE activity depends on synthesis of new enzyme, which requires days to months. Some quaternary OP compounds (e.g., echothiophate) combine at both the esteratic and anionic sites, and that probably contributes to their extreme potency and specificity (4,16).

From the foregoing account, it is apparent that the terms "reversible" and "irreversible," as applied to the carbamyl ester and OP anti-ChE agents, respectively, reflect only quantitative differences and that both classes of drugs react with the enzyme in essentially the same manner as does ACh.

REACTIVATION OF ACHE

Although the phosphorylated esteratic site of AChE undergoes hydrolytic regeneration at a low or negligible rate, Wilson (17) found that the nucleophilic agent hydroxylamine (H_2NOH) can reactivate the enzyme much more rapidly. In the subsequent search for more effective reactivators, a large number of hydroxamic acids

(RCONHOH) and oximes (RCH=NOH) was shown to have this property, e.g., diacetyl monoxime, or DAM. From the data that accrued, it was predicted that highly effective reactivation should be produced by a molecule containing both a quaternary nitrogen atom and an oxime group, spaced at an appropriate distance. This goal was achieved (18) with pryidine-2-aldoxime methyl chloride (2-formyl-1-methylpyridinium chloride oxime, pralidoxime); reactivation with this compound occurs in one millionth the time of that with hydroxylamine (19). Some bisquaternary oximes were later shown to be even more potent as reactivators; an example is obidoxime chloride (Figure 3)--1,1'-(oxydimethylene)-bis(4-formylpyridinium), dichloride dioxime (20).


Diacetyl Monoxime (DAM)

Pralidoxime Chloride (2-PAM)

Obidoxime Chloride

Fig. 3: Cholinesterase reactivators. Reproduced from Koelle, G.B. In:The Pharmacologic Basis of Therapeutics (Goodman, L.S. and Gilman, A., eds.), 5th ed. Macmillan Publ. Co., 1975, pg. 458.

The mechanism of reactivation is sketched in Figure 4. When the quaternary ammonium group of pralidoxime is attracted electrostatically to the anionic site of the enzyme, the oxime group of the former is oriented optimally to exert nucleophilic attack on the electrophilic phosphorus atom of the phosphorylated esteratic site; the oxime-phosphonate is then split off, leaving the enzyme (21,22).

Although the oximes reactivate phosphorylated cholinesterase in vitro and increase recovery from intoxication produced in vivo by some organophosphates, they are not panaceas for the treatment of poisoning by anti-ChEs. Pralidoxime and obidoxime are quaternary ammonium compounds, and their capacity to reactivate brain enzymes is inhibited by the blood-brain barrier. It is also debatable whether pralidoxime and related agents can effectively antagonize the manifestations of intoxication by neostigmine and other carbamyl ester inhibitors. Moreover, most phosphorylated AChEs undergo a fairly rapid process termed "aging" and within the course of minutes or hours, thereby become completely resistant to reactivators.

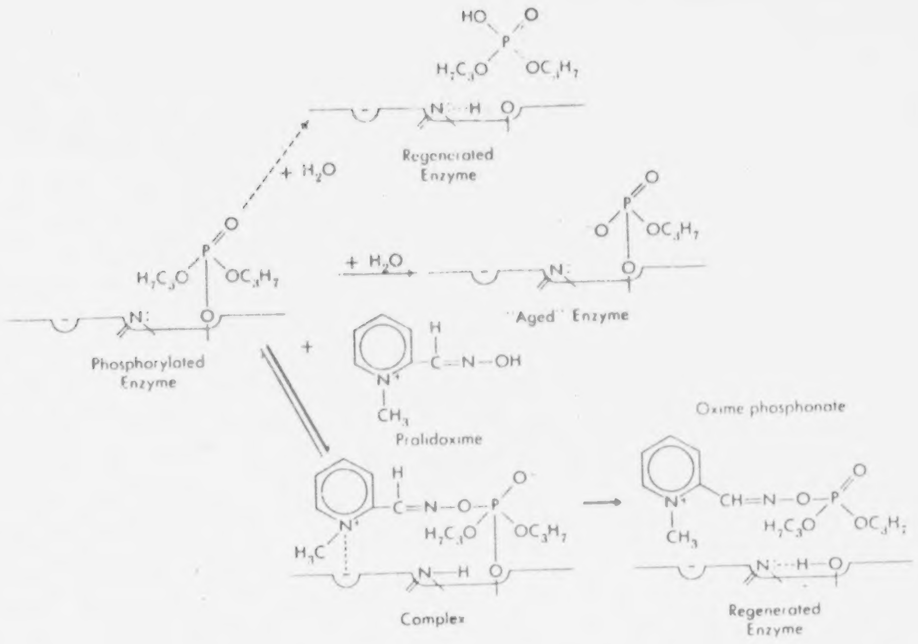


Fig. 4. Reactivation of alkylphosphorylated acetylcholinesterase (AChE). After alkylphosphorylation of AChE by DFP (left), spontaneous hydrolytic reactivation occurs at an insignificant rate (upper reaction), as indicated by the dashed arrow. "Aging" is the loss of one of the isopropoxy residues that occurs more rapidly than spontaneous hydrolysis; the product is very resistant to regeneration by pralidoxime. Pralidoxime (lower reaction) combines with the anionic site by electrostatic attraction of its quaternary nitrogen atom, which orients the nucleophilic oxime group to react with the electrophilic phosphorus atom; the oxime-phosphonate is split off, leaving the regenerated enzyme. Reproduced from Koelle, G.B.: In The Pharmacologic Basis of Therapeutics (Goodman, L.S. and Gilman, A., eds.), 5th ed. Macmillan Publ. Co., 1975, pg. 457.

AGING OF INHIBITED ACHE

Aging causes inhibited enzymes to become refractory to reactivation. The phenomenon was first reported in 1955 by Hobbiger (23) and later observed with OP-inhibited AChEs (24,25) and with an OP-inhibited atropinesterase (26). An extensive discussion of aging may be found in Berend's thesis (27).

The process determines the time during which reactivators can be expected to be beneficial for those exposed to OP anti-ChEs. In the case of OP anti-ChEs, an inhibited (e.g., phosphorylated) enzyme, which initially can be reactivated by oximes, is changed to a form that cannot be reactivated by these compounds (19). The term "aging" has been applied because the amount of inhibited enzyme refractory to reactivation increases with time.

For example, Berry et al. (28) observed that, although pretreatment of animals with a combination of pralidoxime (2-PAM) and atropine increased the LD_{50} values of several compounds (e.g., TEPP and DFP), it had little effect on the lethality of sarin and none on the lethality of soman; they concluded that this was the result of the rapid aging of the ChEs inhibited by sarin and soman. More direct evidence of in vivo aging was obtained by Harris et al. (29), who injected ^{32}P-labeled sarin and soman into rats and observed that the rate of aging of the inhibited rat-brain AChE was the same in vivo as in parallel in vitro experiments.

Aging is probably due to the splitting-off of one alkyl or alkoxy group from the inhibited enzyme, leaving a more stable monoalkyl- or monoalkoxy-phosphoryl AChE (30,31). Wilson (32) pointed out that the difference in reactivatability may be associated with the relative reactivity of secondary and tertiary phosphate esters. Whereas the original inhibited enzyme is a tertiary phosphate ester, the dealkoxylated derivative is a secondary phosphate ester. Thus, the rate of aging depends on the phosphoryl group (33) and not on the group hydrolyzed from the OP by cholinesterase (ChE). Berry and Davies (28) observed, as have many others, that soman yields the most rapidly aged-inhibited ChE obtained from any available anti-ChE; the half-life of the pinacolyl phosphonylated enzyme was determined to be less than 1.5 min. The next most rapidly aged-inhibited enzyme also contained a branched-chain secondary group; $(CH_3)_2$-CH-CH(CH_3)-OH-) Some straight-chain secondary groups, such as CH_3-CH_2-CH(CH_3)-OH-, were also associated with relatively rapidly aged-inhibited enzymes, the phosphonylated enzyme half-life being about 0.5 h. Berry and Davies (28) noted that aging "is slow when the alkyl group is a primary alcohol, whether or not the carbon chain is branched, but is much more rapid if the alkyl group is a secondary or cyclic alcohol."

CHOLINERGIC RECEPTOR AND ACH CHANNEL

In addition to reacting with ChEs, OPs and other anti-ChEs also can react with other critical molecules in nerves or in effector organs. OP drugs may exert direct effects on the cholinergic receptor or on its phospholipid environment, at both CNS and PNS synapses (34-38). OPs also react with other neural and metabolic enzymes, and some of them are capable of alkylating DNA. The biologic consequences of these reactions are not as well understood as are those inhibiting ChE.

White and Stedman (39) suggested that, in addition to inhibiting AChE, OP compounds have an effect on the site where the ACh molecule reacts at the neuromuscular junction. Riker and Wescoe (40) showed a direct agonist action of neostigmine at the neuromuscular junction, and many others have found that neostigmine and some other anti-ChE agents have anticurare effects not apparently related to inhibition of ChE (41). Additional observations indicated that preparations that had been denervated for over 20 d responded with a contracture when exposed to sarin, and findings described below indicate that anti-ChE agents like neostigmine cause marked destruction of denervated muscle (42,43); this phenomenon is most likely correlated with the partial agonist effect of neostigmine.

Similarly, Miquel (44) suggested that OP compounds react with other sites on the muscle, in addition to the enzyme itself. Studies by Xavier and Valle (45) disclosed that Phosdrin^{R}, an OP insecticide, was able to affect both the ACh receptor and the ion channel associated with it, but without affecting AChE itself. They also found, using two different methods, that physostigmine and neostigmine, in addition to producing blockade of AChE, potentiated the muscle response to ACh when applied in the presence of complete AChE blockade. Albuquerque's studies with OP compounds (46) have suggested an additional effect on the ionic channel that is unrelated to the sites of reaction of ACh on AChE or on the ACh receptor. The simple presence of OP compounds makes the reaction of many agonists, particularly ACh and anatoxin (AnTX), more intensive and speeds the effects of some compounds that block the ion channel. Several agents that react both with the ACh receptor and the channel appear to antagonize the action of the OP compounds in this manner. Conversely, the binding rate of OPs is increased when increasing concentrations of the agonist are present (46), and the rate of binding induced by the presence of large quantities of agonist can occur whether ACh is the agonist or other agents--such as AnTX, subaryldicholine, and succinylcholine--are used in place of ACh.

PRESYNAPTIC ACTION

Anti-ChE agents have presynaptic effects that are related mostly to a reaction with the presynaptic nerve terminal (47-55).

A number of workers reported that compounds such as neostigmine and physostigmine augment and prolong spontaneous release of ACh (miniature endplate potentials or MEPPs) and increase the size of endplate potentials (EPPs) (48,56-59). Boyd and Martin (48) reported a biphasic effect of ChE inhibitors: an increase at low concentrations and a decrease at high concentrations. In a detailed study of the action of neostigmine, ambenonium, edrophonium, and methoxyambenonium on cat tenuissimus muscle, Blaber and Christ (60) reported that MEPP frequency was increased by several ChE inhibitors, and concluded that the effect is probably not related to ChE inhibition but might be related to excitation-secretion coupling, the process by which an action potential releases ACh.

The only published study on presynaptic effects of the more potent ChE inhibitors is that of Abraham and Edery (49), who examined the effect of soman on synaptic transmission in rat diaphragm. In vitro, soman increased the frequency of MEPPs (an effect blocked by Mg^{2+}), caused muscle depolarization that reversed spontaneously, and increased quantal content. It was suggested that the observed changes in transmitter release resulted from an effect on the action potential invading the nerve terminal, although no direct evidence was offered.

In addition, several ChE inhibitors generate antidromic action potentials in motor nerves; these may occur spontaneously (57,61, 62) or after an orthodromic nerve volley (47,50,62-64). The potentials are apparently not caused by action potentials originating in muscle, inasmuch as the antidromic repetitive firing has been observed in nerves from muscle incapable of twitching (62). Riker et al. (50) and Werner (51,52) concluded that the antidromic discharges were produced by direct actions on the motor nerve terminal. Although ChE inhibition, with later accumulation of ACh and an increase in extracellular potassium concentration, may be the cause of the observed effects, these possibilities are unlikely.

NERVE MEMBRANE

Another example of an effect apparently unrelated to ChE inhibition is found in studies of the actions of ChE inhibitors on ionic conductances of electrically excitable membranes. When single frog nerve fibers were used, physostigmine (1-10 mM) attenuated action potential and current, markedly prolonged the duration of the current, and slowed conduction. This mechanism might be involved in functional sensory deficit and--if selective for inhibitory fibers, as is the case with local anesthetics (65)--might play a role in generation of facilitation before depression.

CENTRAL NERVOUS SYSTEM

The situation is still more complex in the CNS. Even if the action of anti-ChEs were limited to the inhibition of postsynaptic AChE, the complex circuitry of the brain provides ample opportunity for effects at other sites. Because brain cholinergic pathways are diffuse and connect with many other systems, overactivity or blockade of cholinergic synapses can lead to abnormal activity in many other neurons. Apparently, there is no end to the list of transmitters and bioactive substances that can be affected indirectly or directly by cholinergic agonists (66). Among the effects in question are those on the γ-aminobutyric acid (GABA) system which are important in brain excitability and epileptogenesis (67), as well as those involving peptide transmitters and bioactive peptides (68). It is unknown whether these effects are brief or long-lasting. For example, it is unlikely that a perturbation in GABA content would be long-lived after the initial effect of the anti-ChE on the GABA system. However, the circuits are complex, and even a temporary perturbation might lead to reverberations that persist for a long time. The CNS has only a limited capacity to regenerate, and recovery after tissue damage might be slow or incomplete. It is also known that a brief presence of excess transmitter in a synapse can lead to compensatory changes in the number of postsynaptic receptors.

"NEUROTOXIC" ESTERASE (NTE)

Some OPs react with a poorly characterized enzyme--"neurotoxic esterase" (NTE)--of unknown function resident in CNS, PNS, and some other tissues and precipitate a delayed CNS-PNS distal axonal degeneration, which is expressed clinically as a sensorimotor neuropathy (69). Although a causal association between NTE inhibition and delayed neurotoxicity has *not* been demonstrated, they are strongly correlated.

NTE is operationally defined as the esteratic activity against phenyl phenylvalerate (the preferred substrate), phenyl valerate, or closely related esters that is "resistant" to paraoxon and sensitive to DFP and mipafox (*N*,*N*'-diisopropylphosphorodiamidic fluoride). Two groups of compounds inhibit NTE (Figure 5): one group consists of various phosphates, phosphoramidates, and phosphonates, which induce neuropathy; and the second contains sulfonates, phosphinates, and carbamates, which do not. The latter can react covalently at the phosphorylation site involved in delayed neurotoxicity and thereby protect against later doses of OP compounds that would otherwise induce neuropathy. In contrast, neuropathic OP compounds induce irreversibly (aged) inhibited NTE. Aging involves transformation of the phosphorylated enzyme to a further modified form in which one R group has been cleaved from the phosphorus and a negatively charged residue remains attached to the enzyme. Once this process occurs, regeneration of the active site of the enzyme is no longer possible, and neuropathy will ensue if the NTE-inhibition threshold has been reached or exceeded within a required period. The relationship between this phenomenon and the onset of axonal degeneration is unknown, but it seems likely from recent experimental studies with DFP (70,71) that the target site in nervous tissue is in the nerve fiber itself.

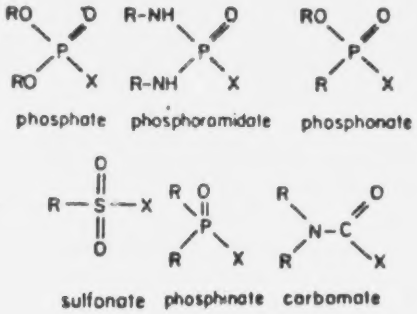


Figure 5. Two groups of NTE inhibitors; Group A (upper) induce delayed neuropathy, Group B (lower) protect against the neuropathic potency of the upper group. R and R' may be alkyl, aryl or heterocyclic substituents; X is the leaving group which is ejected when the inhibitor reacts covalently with the enzyme. Redrawn from Johnson, M.K., 1974: A comparison of the structures of inhibitors of "neurotoxic esterase". J. Neurochemistry 23:786, Fig. 1, Raven Press, N.Y.

BIOCHEMICAL AND METABOLIC CHANGES

OP and related drugs have effects on dehydrogenases and on gluconeogenesis and oxygen uptake (6,72). Failure to gain weight normally accompanies chronic intoxication with some OP agents, and weight loss accompanies OP-induced neuropathy. Some of these biochemical changes may not be specifically related to the anti-ChEs; e.g., changes in blood lactate and acid-base relationships could be related to severe hypoxia, and the hyperglycemia could result from discharge of catecholamines via cholinergic control of the adrenal medulla.

OP ALKYLATION

Some organophosphates are strong alkylating agents in vitro (73,74). Examples are tetramethyl pyrophosphate, dichlorvos, methyl paraoxon, tetrachlorfenvinphos, mevinphos, crotoxyphos, and methyl parathion. Review of several studies on the alkylation of DNA with dichlorvos indicated that dichlorvos induced methylation in isolated DNA or in DNA of bacterial and animal cells treated in vitro. The extent to which OP esters alkylate DNA in vivo is not clear. On the one hand, the existence in mammals of considerable extrahepatic esterase activity provides an important detoxifying capability that diminishes the chances of deleterious alkylation (74,75). On the other hand, it was reported (76) that [^{14}C]parathion administered in the diet or by intraperitoneal injection resulted in binding of metabolites to liver DNA. It was also assumed that the esterase detoxification pathways may operate only slowly, or not at all, for simple alkyl phosphates like trimethyl phosphate (74,75).

BIOLOGIC AND CLINICAL EFFECTS

ACUTE EFFECTS: PHARMACOLOGY

The characteristic acute pharmacologic effects of the anti-ChE agents classically are ascribed to the inhibition or inactivation of AChE at sites of cholinergic transmission, with the consequent accumulation and action of endogenous ACh liberated both by stimulated cholinergic nerve and (in much smaller amounts) by continual leakage during the resting stage. AChE is present in most tissues in a quantity in excess of that required for normal function; to exert a marked effect *in vivo*, an anti-ChE agent must generally inhibit 50-90% of the functional AChE at a given site. This can be achieved readily, because most of the anti-ChEs produce 50% inhibition of the enzyme at concentrations of 10^{-7} M or lower.

In principle, it should be possible to predict the pharmacologic properties of anti-ChE agents merely by knowing the loci at which ACh is physiologically released by nerve impulses, and the responses of the corresponding effector organs to the chemical mediator. Potentially, the anti-ChE agents can produce all the following effects: stimulation at autonomic parasympathetic effector organs; stimulation, followed by depression or paralysis, of skeletal muscle and of all autonomic ganglia (nicotinic actions); and stimulation, followed by depression, of cholinoceptive sites in the CNS. Because cholinergic stimulation of ganglia increases activity in the sympathetic, as well as the parasympathetic, postganglionic nerves, all the autonomic effectors are activated.

These assumptions are only broadly correct, inasmuch as, with smaller doses of anti-ChEs, particularly those used therapeutically, several modifying factors are present. Most importantly, an enormous number of compounds can inhibit cholinesterase, and no two are identical in either biochemical properties or pharmacokinetics. Thus, although they all share some general characteristics, the detailed effects vary considerably from compound to compound. For example, compounds containing a quaternary ammonium group do not penetrate cell membranes readily; hence, some anti-ChE agents are excluded by the blood-brain barrier from exerting substantial action on the CNS. But quaternary ammonium compounds act relatively strongly at the neuromuscular junctions of skeletal muscle through both their anti-ChE and their direct cholinomimetic mechanisms and have comparatively less effect at autonomic effector sites. Their ganglionic actions are generally intermediate. The more lipid-soluble agents, such as tertiary amines and most OP compounds, have ubiquitous effects at both PNS and CNS cholinoceptive sites.

The main acute pharmacologic actions of anti-ChE agents that are of concern here are those on the eye, the intestine and other organs innervated by the autonomic division of the PNS, the skeletal neuromuscular junction, and the brain. Effects of cholinergic and adrenergic stimulation on effector organs are summarized in Table 1.

Eye

When applied to the conjunctiva, anti-ChE agents cause conjunctival hyperemia and constriction of the iris sphincter (miosis) and ciliary muscle (spasm of accommodation). Miosis is apparent in a few minutes and becomes maximal in 0.5 h. The pupil may be pinpoint sized, but it generally contracts even further when exposed to light. It returns to its normal size in a few hours to several days, depending on the drug and its concentration. The spasm of accommodation is more transient and generally wanes considerably before termination of the miosis. Intraocular pressure usually decreases concomitantly, but in some cases anti-ChE agents may cause an initial increase in intraocular pressure owing to dilatation of the finer blood vessels and increased permeability of the blood-aqueous humor barrier; this is generally followed by a decrease to below initial pressure.

Systemically administered anti-ChEs similarly affect the cholinergic terminals supplying the circular muscles of the eye to produce miosis, but they also affect sympathetic ganglia that operate the apposing radial muscles, and they act on the brain in ways that may reduce activity in cholinergic nerves to the eye. Thus, the effects of systemic administration are not as predictable as those of local administration, and either constriction or dilatation of the pupils may be seen.

Gastrointestinal Tract

Although the actions of various anti-ChE agents on the gastrointestinal tract are nearly identical, neostigmine has been studied most extensively in this regard. In man, neostigmine increases gastric contractions and increases the secretion of acidic gastric juice. The drug tends to counteract the inhibition of gastric tone and motility induced by atropine and increases the stimulatory effect of morphine.

Neostigmine augments the motor activity of the small and large bowel; the colon is particularly stimulated. Atony is overcome or prevented, propulsive waves are increased in amplitude and frequency, and transport is thus promoted. Atropine inhibits, but does not abolish, the intestinal effects of neostigmine. The total effect of anti-ChE agents on intestinal motility probably represents a combination of actions at the ganglion cells of Auerbach's plexus and at the muscle fibers, as a result of the preservation of ACh released by the cholinergic preganglionic and postganglionic fibers, respectively.

Actions at Other Autonomic Sites

Secretory glands that are innervated by postganglionic cholinergic fibers include the bronchial, lacrimal, sweat, salivary, gastric, intestinal, and acinar pancreatic glands; low doses of anti-ChE agents cause, in general, an augmentation of their secretory responses to nerve stimulation, and higher doses increase the resting rate of secretion.

Smooth muscle fibers of the bronchioles and ureters are contracted by these drugs, and the ureters may show increased peristaltic activity.

The cardiovascular actions of anti-ChE agents are extremely complex, in that they reflect at any given moment the sum of the excitatory and inhibitory actions of accumulated endogenous ACh at several levels. The predominant cardiac effect of the peripheral action of accumulated ACh is bradycardia, which results in a decrease in cardiac output and in hypotension. The effective refractory period of cardiac muscle fibers is shortened, and the refractory period and conduction time of the conducting tissue are prolonged. The blood vessels are in general dilated, although the coronary and pulmonary circulation may show the opposite response. The sum of the foregoing effects should result in hypotension, but at the ganglionic level ACh has first an excitatory and, at higher concentrations, an inhibitory action. Hence, the excitatory action on parasympathetic ganglion cells tends to reinforce the above effects, whereas the opposite sequence results from the action of ACh on sympathetic ganglion cells. Excitation followed by inhibition is also produced by ACh at the medullary vasomotor and cardiac centers. All these effects are further complicated by the hypoxia resulting from bronchoconstriction and other actions on the respiratory system. The hypoxia reinforces both sympathetic tone and ACh-induced discharge of epinephrine from the adrenal medulla. It is not surprising, therefore, that a wide variety of hemodynamic effects of anti-ChE agents has been reported, depending on drug, dose, route of administration, species, and other factors.

Neuromuscular Junction

The actions of anti-ChEs are thought to be due to inhibition of ChE at the motor endplate and retention of ACh at the junctional region. This combination culminates in a marked reaction of the transmitter with the receptor and a great activation of the entire receptor-ion-channel complex. If this process continues, a number of undesirable reactions can occur, among them paralysis, the desensitization of the junctional receptor, and structural changes in the muscle and nerve ending.

Normally, a single nerve impulse in a terminal motor axon liberates enough ACh to produce a localized depolarization (the endplate potential) that initiates a propagated muscle action potential. The liberated ACh is rapidly hydrolyzed by AChE, and the muscle relaxes. Therefore, each motor-nerve impulse initiates only one muscle contraction. After partial inhibition of AChE, however, the ACh liberated by a single nerve impulse may persist long enough to set up repetitive muscle action potentials, with a resulting increase in strength of contraction. Furthermore, sufficient ACh may diffuse to neighboring muscle fibers and excite them as well, causing asynchronous contractions (fibrillation). In addition, the action of anti-ChE agents on the axon terminal can initiate antidromic firing, which results in activation of the motoneuron and leads in turn to the synchronous contraction of an entire motor unit (fasciculation). In the presence of a sufficiently high dose of an anti-ChE agent, the local concentration of ACh may produce a depolarizing blockade of the neuromuscular junction and paralysis. Thus, a small dose of anti-ChE may increase the skeletal muscle contraction produced by a single maximal nerve stimulus, but larger doses, or repetitive nerve stimulation at a high physiologic rate, may result in depression or block of neuromuscular transmission.

Brain

The mechanisms by which anti-ChEs perturb brain function are more complex, harder to study, and consequently less well understood. The brain is an extraordinarily complex network of neurocellular pathways which uses electrochemical mechanisms to conduct signals needed to perform and integrate cognition, awareness, memory, language, sleep and wakefulness, locomotion, sensation, and hormonal and autonomic functions. Cholinergic neurons are probably involved in many intraregional and interregional pathways of the brain, although their identity and specific functions are poorly understood. Several sets of presumptive central cholinergic pathways have been proposed: medial septal nucleus to dentate gyrus, and subiculum of hippocampus habenula to interpeduncular nucleus; cortical internurons to cortical pyramidal neurons; and thalamus, putamen, and caudate to neurons in the caudate (77). The presence of cholinergic synapses in central motor pathways (pyramidal and extrapyramidal) and of afferent systems involving both the reticular formation and the thalamus, hippocampus, and limbic system, suggests the possibility of their participation (with other types of chemical synapses) in initiation and control of movement, in sleep, arousal, and wakefulness, in memory, and in emotional regulation. This, in turn, implies susceptibility of these functions to anti-ChEs and cholinomimetic drugs.

The electroencephalogram (EEG), a record of changes in the voltage-field distribution over the head as a function of time, is a tool for studying some aspects of brain function. Adults have characteristic EEG patterns under standard conditions, but these vary according to the state of consciousness (alert, startled, drowsy, dreaming, or deeply sleeping). Awake subjects display a high-voltage (50 μV), low-frequency (8-14 Hz) alpha rhythm. This is most prominent in the occipital region of the scalp and when the eyes are closed. The resting alpha rhythm is replaced during periods of attention and problem-solving by a low-voltage (5-10 μV), high-frequency (15-30 Hz) beta rhythm, most prominent in frontal and parietal regions. The changeover from alpha to beta rhythm, termed "desynchronization", can be induced by mental concentration or external stimuli (including anti-ChEs). Other frequencies of electric activity commonly observed in the EEG are theta (4-7 Hz) and delta ($\leq$ 3 Hz) waves.

Among brain functions, sleep is particularly well studied with the EEG. When sleep occurs, the alpha pattern disappears and, over a period of 4-5 min, the EEG changes from a low-voltage to a higher-amplitude, 4- to 6-Hz pattern with intermittent 14- to 16-Hz "spindle" activity. Later, over the course of 1-2 h, the voltage increases, the frequency decreases (to 1-3 Hz), and spindle activity becomes less frequent. The EEG then becomes desynchronized, rapid eye movement (REM) occurs, and the person dreams. REM sleep lasts approximately 15-20 min and occurs three to five times during a normal sleep cycle of 7-8 h. These characteristics are constant from night to night if the person is healthy, is well adapted to the environment, and has an habitual, normal, 24-h sleep-wake cycle.

The control of the sleep-wake cycle appears to be a complex phenomenon involving several groups of neurons (nuclei) in the brainstem that use different synaptic transmitter chemicals: the locus ceruleus (norepinephrine), the dorsal raphe nuclei (serotonin), and the gigantocellularis nuclei (ACh) of the pontine reticular formation (78). Cholinergic neurons in the gigantocellularis nuclei concentrate their electric discharges during REM sleep: activity begins minutes before the onset of REM sleep, continues at a high rate during the REM period, and abruptly ceases as REM sleep terminates. An attractive postulate of the control of the sleep-wake cycle is that, during non-REM sleep (and waking), an inhibitory system composed of neurons in the locus ceruleus or the dorsal Raphe nuclei tonically prevents cholinergic neurons in the gigantocellularis nuclei from firing. At a critical point, the latter escape this inhibition and fire at very high rates, thereby initiating the REM period. The inhibitory neurons then increase their activity and inhibit the cholinergic discharge, and REM sleep ceases. Dependence on ACh as the excitatory transmitter implies the sensitivity of this system to anti-ChEs, which, a priori, tend to increase neuronal activity. This postulate is consistent with empirical evidence from humans and animals treated with cholinergic agonists, which tend to decrease the latency of REM sleep and increase the number of REM-sleep episodes. Atropine exerts opposite effects.

Table 2 lists some of the effects of anti-ChEs and cholinomimetic drugs on brain function (79-84). Doses of OP compounds that are toxic, but too small to threaten life, produce a variety of clinical manifestations, including miosis, muscular fasciculation, and apprehension (85,86). The acute behavioral alterations are usually accompanied by marked desynchronization of the EEG (87). Larger doses of OP compounds--which may induce convulsions, muscular paralysis, and death--cause slowing of the EEG pattern followed by the appearance of spike waves that herald the onset of seizures. Symptomatic recovery is normally complete within 2-9 wk, at which time the erythrocyte cholinesterase content usually has returned to normal (88,89).

Repeated low-dose administration of OP compounds can produce symptoms and signs that are not seen after single exposures to the same doses. For example, subjects given daily injections of DFP reported the additional symptoms of insomnia, excessive dreaming, emotional lability, increased libido, paresthesias, visual hallucinations, and tremor (90); and prolonged administration in animals induces sensorimotor neuropathy.

The subjective manifestations of brain dysfunction usually disappear shortly after cessation of exposure; in most instances, EEG abnormalities reportedly disappear within 2 wk of acute exposure or termination of chronic exposure (90-92).

ACUTE TOXIC EFFECTS

Toxicity of particular OP compounds does not vary greatly among mammals. Signs and symptoms differ mainly in sequence and in individual prominence (93-96). Rapidity of appearance, intensity, and timecourse depend on dose and route of administration.

The effects of acute intoxication with anti-ChE agents are manifest by muscarinic and nicotinic signs and symptoms and, except for compounds of extremely low solubility in lipids, signs referable to the CNS. Effects may be local or general. Local effects are due to the action of vapors or aerosols at their site of contact with the eyes or respiratory tract or to the local absorption after liquid contamination of the skin or mucous membranes, including those of the gastrointestinal tract. General effects rapidly follow systemic absorption by any route; they appear most rapidly after inhalation of vapors or aerosols, in which case severe effects may appear within a few minutes. In contrast, the onset of symptoms after gastrointestinal and percutaneous absorption is delayed. The duration of effects is determined largely by the nature of the compound; it may vary from minutes, as after an overdose of edrophonium, to several days or even weeks after irreversible alkylphosphorylation of AChE, as by DFP or sarin.

After exposure to vapors or aerosols or after inhalation, ocular and respiratory effects generally appear first. Ocular effects include marked miosis, conjunctival congestion, ciliary spasm, and browache, along with watery nasal discharge; respiratory effects consist of "tightness" in the chest and wheezing due to the combination of bronchoconstriction and increased bronchial secretion. After ingestion, gastrointestinal effects appear first, including anorexia, nausea and vomiting, abdominal cramps, and diarrhea. After percutaneous absorption of liquid, localized sweating and muscular fasciculation in the immediate vicinity are generally the earliest manifestations. Severe intoxication is manifest by extreme salivation, involuntary defecation and urination, sweating, lacrimation, bradycardia, and hypotension.

Nicotinic actions at the neuromuscular junctions of skeletal muscle usually consist of fatigability and generalized weakness, involuntary twitching, scattered fasciculation, and eventually severe weakness and paralysis. The most serious consequence of the neuromuscular actions is paralysis of the respiratory muscles.

The effects on the CNS include confusion, ataxia, slurred speech, loss of reflexes, coma, and central respiratory paralysis. Actions on the vasomotor and other cardiovascular centers add to the peripheral actions to complicate the hemodynamic pattern. After large doses or inhalation of high concentrations, the time course may be telescoped into a few minutes and many of the above signs overshadowed by dyspnea, apnea, and collapse. A case of severe accidental poisoning in man is illustrative (97). Development of signs after smaller doses has been described by Grob (98,99) and others (100-104).

After a single exposure, death may come within 5 min or not for some 24 h, depending on dose, route of administration, drug, and other factors. The cause of death is primarily respiratory failure, usually accompanied by a cardiovascular component. Muscarinic, nicotinic, and central effects all contribute to respiratory embarassment; they include laryngospasm, bronchoconstriction, increased tracheobronchial and salivary secretion, and peripheral and central respiratory paralysis. Although the blood pressure may fall alarmingly and cardiac irregularities may intervene, these effects probably result as much from hypoxia as from the specific actions mentioned, inasmuch as they can often be reversed by the establishment of adequate pulmonary ventilation.

Differences in hazard among members of the OP group arise from differences in inherent potency (EA 3148 is the most potent), vapor pressure (GB is hazardous by inhalation), and ability to penetrate the skin (VX and GD) (102,105,106). GB as a liquid administered cutaneously has also been reported to cause severe poisoning (107, 108). Absorption of some of these compounds from the respiratory tract has been estimated to approach absorption after intravenous administration in completeness (105,109,110). Incapacitating concentrations of GB and others by inhalation have been estimated in animal tests, (111) and extrapolations to man have been attempted (105,112,113).

Two antidotes are currently used for acute poisoning by anti-ChEs. One is atropine, which acts as a muscarinic receptor antagonist and reduces the excessive stimulation of parasympathetic functions, thus reversing the effects on the eye, lung, gastrointestinal muscles, and, most important, the heart. Atropine also relieves effects of poisoning at central muscarinic synapses, but not at central nicotinic synapses. The other antidote is an oxime (such as 2-PAM or obidoxime) that relieves poisoning at skeletal muscle endplates (20). It acts as an activator of phosphorylated AChE; through nucleophilic attack, it removes the phosphate group, thus restoring enzyme function at motor endplates. The oximes are inefficient if the phosphorylated enzyme has aged. These oximes contain quaternary nitrogen and therefore do not penetrate the blood-brain barrier and have no effect at cholinergic synapses in the brain or spinal cord.

The acute effects of the anti-ChEs are short-lived and do not outlast the inhibition of the enzyme. Indeed, some systems develop tolerance rapidly, so function returns to normal even before there is substantial regeneration of measurable enzyme activity. It has been amply documented that, even with over 99% inhibition of all ChEs, animals (and presumably humans) can survive without oxime or atropine treatment, if they are supported for a couple of hours by pharmacologic or nonpharmacologic means, such as artificial respiration, (72). This recovery from the "irreversible" effects of inhibitors may depend on rapid regeneration of ChEs, particularly some AChE isoenzymes (114); desensitization of the postsynaptic membrane, a phenomenon that limits the response to accumulated ACh; or compensatory changes in presynaptic and postsynaptic receptors (115).

DELAYED NEUROPATHY (CNS-PNS DISTAL AXONOPATHY)

Clinical Features

Degeneration of particular regions of the nervous system is a well-characterized adverse health effect of human and animal exposure to many OP esters (phosphates, phosphoroamidates, and phosphonates) that may or may not also display anti-AChE properties. Some neuropathic OP esters can precipitate prominent neurologic abnormalities after a single exposure (as well as after multiple exposures), the clinical disease usually beginning within 2-3 wk. At some time during this clinically quiescent period, a stereotyped sequence of neuropathologic changes takes place that leads to the appearance of sensorimotor neuropathy. The degree of clinical impairment and the prognosis for functional recovery depend directly on the extent of nervous system damage, which in turn depends on the neuropathic potency of the responsible OP compound, as well as the dose and duration of exposure.

The first recorded cases of paralysis from OP intoxication occurred at the end of the nineteenth century, when patients with tuberculosis were treated with phosphocreosote, an uncharacterized mixture of esters derived from phosphoric acid and coal-tar phenols (116). Several thousand cases appeared in the southern states in 1930 when alcoholic extracts of Jamaica ginger, widely consumed during Prohibition, were adulterated (117) with 2% triorthocresylphosphate (TOCP). Adulteration of cooking oil contaminated with lubricating oil containing cresyl phosphates has proved responsible for several outbreaks of OP neurotoxicity, including a major epidemic in Morocco in which more than 10,000 people reportedly were affected (118). More recently, the OP pesticide leptophos has been associated with an outbreak of occupational neurotoxicity among workers at a plant in Texas; the victims displayed pronounced clinical features of spinal-cord damage, and some had psychologic manifestations (119).

Approximately 2 wk after ingesting cresyl phosphates, during which gastrointestinal disturbances may be manifest, affected persons experience pain, aches, and tingling in the feet and calves, followed within days by progressive weakening of leg and foot muscles that leads to paralysis. The thighs and then the hands and arms may become weak during succeeding days. Weakness is always more severe in the legs than in the arms and in both limbs is greatest in distal muscles. During the progressive phase of the illness, which lasts 1-2 wk (depending on dose), the weakness spreads steadily, but usually stops short of complete quadriplegia. Neurologic examination reveals signs of damage to the spinal cord (hyperactive knee jerks) and peripheral nerves (hypoactive ankle jerks). Foot drop is pronounced, and victims adopt a high-stepping gait. Muscle denervation is evident from electromyography, and atrophy in the lower legs and hands may become severe. Recovery in mild cases takes months or years, but severely affected persons who recover some muscle strength may have ataxia and spasticity permanently (120).

Many experimental species are vulnerable to the delayed neurotoxic effects of OP compounds, such as TOCP, although it is accepted that neurotoxic doses vary markedly from one species to another. Rodents are relatively resistant and fowl very susceptible; hens are widely used to assay AChE compounds for ability to induce paralysis (121). In all sensitive species, there is a period of 1-2 wk before the onset of neurologic signs, during which body weight changes and nerve fibers degenerate. Hens given TOCP develop a steadily increasing flaccid paresis of the hindlimbs, with an ataxic, broad-based gait. Paresis spreads over the course of several days and, if respiratory muscles become involved, may lead to death.

Neuropathologic Features

The limited neuropathologic information available from studies of affected persons demonstrates that OP poisoning induces degeneration of nerve fibers in spinal cord and peripheral nerves (122).

Neuropathologic examination of experimentally poisoned animals reveals a characteristic pattern of distal, retrograde degeneration of peripheral nerves. Long nerve fibers of large diameter seem to be affected before shorter and smaller fibers, so sensory and motor manifestations of nerve damage generally affect the legs before the arms. A similar principle holds for involvement of the spinal cord: long ascending (gracile and spinocerebellar) and long descending (e.g., corticospinal) tracts are symmetrically involved in the degeneration process. Neuropathologic changes initially appear distally and multifocally in affected pathways, leading to degeneration of distal axons and structural and functional disconnection of sensory and motor terminals. Axonal degeneration progresses steadily toward, but stops short of, the nerve cell bodies (71). Loss of axons precipitates a secondary loss of the normal myelin sheath in affected regions of spinal cord and peripheral nerves; this phenomenon has been erroneously described as "demyelination"--a term reserved to describe the neurotoxic properties of substances, such as hexachlorophene, that damage myelin without causing axonal degeneration. In sum, the clinical and pathologic features of delayed OP neuropathy are classified as a central-peripheral distal axonopathy (123).

Neuropathic Potency

Neuropathic potency can be assayed by determining the response of a vulnerable species (the hen, *Gallus gallus domesticus*) to OP intoxication or predicted from the degree of inhibition of the nervous-system enzyme NTE. It is important to note that chemical reactivity of an OP compound with NTE is *unrelated* to its ability to inhibit AChE (69). Some OP agents designed for chemical warfare can inhibit both NTE and AChE. However, doses needed to inhibit NTE and induce neuropathy may be much higher than those which would prove fatal to animals or humans without protection against the acute anti-ChE effects. Alternatively, the degree of NTE inhibition may be insufficient to induce clinical neuropathy. Phosphorofluoridates induced delayed neuropathy in chickens after 9-15 d at doses of 0.3-2.5 mg/kg; the dimethyl compound required doses of 30 mg/kg. Five alkylphosphorofluoridates were active at 1-5 mg/kg given in divided daily doses. Diethyl phosphofluoridothionate induced neuropathy at 0.75 mg/kg, but not at 0.5 mg/kg. Various dialkylphosphinic fluorides and dialkylpyrophosphonates were negative (124). Neuropathologic studies designed to detect subclinical damage to spinal cord or peripheral nerves in animals treated with CW agents are not available.

LONG-TERM BRAIN DYSFUNCTION

Several studies have suggested that some subjects experience long-term sequelae from a single OP exposure or a period of low-level exposure. Minor disorders of affect, emotion, and memory were reported by Tabershaw and Cooper (125) in 38% of 114 subjects after acute OP poisoning. Rowntree *et al.* (126) suggested that OP exposure might exacerbate psychiatric problems. Metcalfe and Holmes (82) claimed that OP exposure may lead to persistent EEG changes; they also reported that workers with histories of both OP and chlorinated-hydrocarbon exposure, but with no recent exposures, had EEG patterns that showed excessive slowing during drowsiness and after hyperventilation. Moreover, all-night-sleep EEGs reportedly displayed patterns commonly associated with narcolepsy. Psychologic dysfunction in this group included disturbed memory and difficulty in maintaining alertness and appropriate focusing of attention.

Duffy and co-workers (127) examined the brain electrical activity of workers occupationally exposed to sarin and with documented single or repeated accidental exposure to toxic concentrations of it at least a year before EEG recording. Standard clinical EEG measurement, computer-derived EEG spectral analysis, and standard overnight-sleep EEGs, were examined in 77 exposed workers, and the results were compared with those from a control group of 38 nonexposed industrial workers. Statistically significant group differences in sarin workers included increased beta activity, delta and theta slowing, decreased alpha activity, and increased REM sleep.

The results of Duffy *et al.* were consistent with those from a previous study that examined EEG changes in rhesus monkeys exposed to sarin or dieldrin (87). Two dose schedules were used: a single "large dose" (sarin at 5 µg/kg or dieldrin at 4 mg/kg, administered intravenously), which produced overt signs of toxicity, and a series of 10 weekly "small doses" (sarin at 1 µg/kg or dieldrin at 1 mg/kg, administered intramuscularly). The effects of anoxia in the first group were precluded by pretreating animals with gallamine triethiodide and providing artificial respiration. Animals treated with single doses of either sarin or dieldrin displayed significant increases in the relative amount of beta voltage (15-50 Hz) in the EEG that persisted for a year. For sarin, the predominant effect was in the EEG derivation from the temporal cortex, and for dieldrin, from the frontal cortex. For both drugs, the increase in beta activity was most prominent when subjects were awake in darkness or drowsy.

In summary, research results have indicated that a single symptomatic exposure or a series of subclinical exposures to sarin can alter the frequency spectrum of the spontaneous EEG for up to a year (83).

The effect of anti-ChEs on the structural integrity of brain tissue has rarely been investigated, although the destructive effects of some of these compounds on spinal cord and peripheral nerves are well known. There is a clear need to study, with current ultrastructural techniques, the possibility of selective brain damage underlying the reported long-term changes in EEG patterns of animals and humans exposed to anti-ChEs, especially in light of a recent provocative report of widespread axonal and terminal degeneration in the brains of rats treated with soman. As an incidental finding in an unrelated study of the effects of this agent on behavior of rats, Petras (128) described nerve-terminal degeneration in the limbic system, corticofugal system, and central motor system-areas associated with mood, affect, judgment, emotion, posture, and locomotion. The author pointed out that damaged regions would be unlikely to regenerate and that long-term psychiatric and motor deficits might be anticipated. Only 16 animals were studied by Petras, and only seven brains had observable damage. The seven had significant acute toxicity at the time of exposure, including muscle fasciculations, tremors, and seizures. Only an abstract of this work has been published, and the research has not been pursued systematically. It cannot now be concluded that the tissue damage was a direct effect of the OP, rather than an indirect effect (e.g., related to brain hypoxia). Nor can it be ascertained whether the response was specific for soman.

JUNCTIONAL NEUROMYOPATHY

Pathologic changes develop subacutely and reversibly in some motor-nerve terminals, neuromuscular junctions, and associated muscle fibers after administration of anti-ChE drugs to laboratory animals. ChE inhibitors with long-lasting effects-such as paraoxon, DFP, tabun, sarin, soman, and parathion-and "reversible" inhibitors, such as physostigmine and neostigmine, induce these neuromyopathic changes (129-131). The effect may result not only from AChE inactivation, but also from an increase in the rate of spontaneously released ACh secondary to a prejunctional action of anti-ChE drugs (54,132). Thus, guanidine, which increases ACh release, also causes a subacute myopathy similar to that produced by anti-ChE agents (133).

The diaphragm is most severely affected in treated animals (134), but abnormalities are also prominent in the soleus, gastrocnemius, and quadriceps muscles. Ultrastructural changes may appear within hours of drug administration, progress over a period of a few days, and resolve within a couple of weeks. Pathologic changes in motor-nerve terminals, neuromuscular junctions, and muscle fibers are associated with an initial decrease in contractile strength after a few days and then a return to nearly normal strength after several more days (135). These pathophysiologic events may be clearly delineated from the degeneration of motor-nerve terminals and atrophy of muscles that follow administration of agents that induce delayed neuropathy (131), in that subacute neuromyopathic changes not only resolve before the onset of delayed retrograde axonal degeneration, but also fail to develop with TOCP, an O-P compound that inhibits NTE but has little AChE activity. They may also be prevented by protecting the neuromuscular junction with curare or a reactivator of phosphorylated ChE, such as 2-PAM. The myopathic process seems to depend on the degree and duration of ChE inhibition (136); this suggests that skeletal-muscle hyperactivity is causally associated with the phenomenon.

The significance of these observations for humans exposed to OP agents is unknown, but it seems likely from animal experiments that myopathy does not develop in the absence of muscle hyperactivity induced by anti-ChEs. There is some evidence from human autopsy material of focal necrosis of diaphragmatic and intercostal muscles after accidental exposure to a single large dose of OP insecticide (137).

Motor-nerve terminals show various degrees of subcellular changes within 30 min to 2 h after injection of soman or paraoxon (138); soman induces the more severe changes. Nerve terminal alterations include the appearance of intra-axonal myelin figures, membrane enclosures, and an increased number of large-coated vesicles. Three days after DFP injection, soleus motor-nerve terminals are reduced in number and naked endplates are common. Pathologic changes also appear in the subneural apparatus and in the immediate subjacent muscle; the latter displays swollen mitochondria, myelin figures, enlarged nucleoli, dilatation of the sarcoplasmic reticulum, loss of myofibrillar striation, and, later, myofilament loss and fragmentation of Z bands (132). Focal muscle necrosis then ensues. Prejunctional and postjunctional subacute changes are resolved within 2 wk after administration of DFP; however, because this compound also inhibits NTE and induces delayed neuropathy, a week after recovery from the subacute neuromyopathic changes motor-nerve terminals undergo a second phase of degeneration and regeneration, with reinnervation of damaged endplates 6-8 wk later (131).

OTHER ADVERSE HEALTH EFFECTS

Mutagenic or carcinogenic action is not a general feature of the OP compounds, although some may have these properties. There appears to be a lack of information on the mutagenicity or carcinogenicity of carbamate anti-ChEs.

Some agents on the list of compounds under scrutiny belong to chemical classes other than OP compounds and carbamates. Edrophonium is a quaternary ammonium inhibitor of ChE. Methacholine is a stable choline ester and, although urecholine is a carbamate, it is not hydrolyzed by ChE. These two compounds are direct-acting cholinomimetic agents. They are approved for use in clinical medicine, but have few indications. Hexafluorenium is a bisquaternary ammonium distantly related to tubocurarine, and it has both anti-ChE and curariform actions. None of these quaternary ammonium compounds is known to have genotoxic action. Another compound is tacrine, 9-amino-1,2,3,4-tetrahydroacridine; as a class, acridines are notorious for mutagenicity and carcinogenicity, but this particular chemical has been used in clinical practice for more than 20 yr without known sequelae.

Mutagenicity

The OP malathion has been investigated extensively in a number of mutagenesis test systems. Some studies were done with a metabolic activating system, some without (malathion requires metabolic activation for its AChE-inhibiting effect). Malathion also has been tested in somatic cells; tests for chromosomal aberrations in human hematopoietic cell lines, sister chromatid exchange (SCE) in human fetal fibroblasts, and the micronucleus test in mice have been performed. A mouse dominant-lethal mutation assay has also been carried out with malathion, although the study was compromised, in that the dose (300 mg/kg) was well below that which is maximally tolerated. Among these studies, only one (reported in an abstract) claimed positive findings (139). These occurred in the mouse micronucleus and SCE tests and in a host-mediated Salmonella assay. The findings concerning the mutagenicity of malathion are therefore equivocal.

A 1975 review (140) concluded that the OPs bidrin, dichlorvos, dimethoate, methyl parathion, and oxydemeton methyl, were mutagens in a single microbial mutation assay. In addition, trimethyl phosphate is a clear-cut mutagen in male mouse germ cells; six other OP compounds (bromophos, diazinon, fenitrothion, malathion, parathion, trichlorphon) apparently are not. Results of assays in four microbial test systems were inconclusive for several OPs (141). Further study of these compounds has not been undertaken.

Carcinogenicity

Malathion given in the diet for 103 wk was not carcinogenic in male and female rats, although the dose used may have been less than that which is maximally tolerated. No increased tumor incidence could be found in mice given malathion for 80 wk.

An International Agency for Research in Cancer (IARC) monograph reported strong evidence of the carcinogenicity of trimethyl phosphate (142) in one species and evidence of carcinogenicy in another. There is equivocal evidence of the carcinogenicity of parathion and dichlorvos in animals. A single dose of dichlorvos reportedly damages the germinal epithelium of mouse testes (143).

With the exception of urethane (ethyl carbamate), a potent mutagen and established carcinogen in laboratory animals (144,145), carbamate compounds have been largely unstudied.

Fetal and Teratogenic Effects

Parathion reportedly increases resorption and lowers the weight of rat fetuses (146). Results for malathion are questionable (147). No data appear to be available on the potential mammalian teratogenicity of military nerve agents.

Effects on Male Reproduction

A single dose of dichlorvos reportedly damages the germinal epithelium of mouse testes severely (143).

Cataractogenic Effects

The introduction of DFP and other long-acting anti-ChE agents in the late 1940s appeared to represent an important advance in the treatment of open-angle glaucoma. These drugs maintained control of intraocular tension when administered frequently and were effective in cases that were no longer controllable with the shorter-acting compounds. Then, in 1966, independent reports simultaneously implicated the long-acting anti-ChE agents in the causation of lenticular opacities (148, 149). The incidence of this effect appeared to be as high as 50%; its development varied directly with the strength of the solution, frequency of instillation, duration of therapy, and age of the patient. With physostigmine and other short-acting anti-ChE agents, the occurrence of lenticular opacities appeared to be no greater than that in nontreated subjects in the same age groups.

The mechanism of the cataractogenic action of the long-acting anti-ChE agents has remained obscure (150). It has been shown that vervet monkeys can serve as a model for the production of an identical picture with the daily, long-term instillation of echothiophate (151). In an extensive series in which children were treated for accommodative esotropia (strabismus) with the instillation of short- and long-acting anti-ChE agents, no lenticular opacities or other serious effects were detected (152).

Hemocoagulation

Aberrations of the clotting mechanism attend exposure to OP anti-ChEs (153). Study of 31 persons exposed to sarin or parathion revealed a biphasic reaction consisting of rapid coagulation immediately after exposure, followed by a prolongation of clotting time (91,154). Anti-ChEs also increase fibrinolysis. Fibrinolysis and hypercoagulability were noted in two patients with mevinphos poisoning (155). Increased coagulation was associated with increased prothrombin activity (secondary to increased Factor VII activity) and with increased prothrombin consumption. Coagulation abnormalities normalize several weeks after exposure, and there is no overt liver damage.

IMMEDIATE AND DELAYED EFFECTS IN VOLUNTEERS AFTER SINGLE OR REPEATED EXPOSURE TO ANTI-CHES AT EDGEWOOD

According to the available information, 1,406 subjects were tested with 16 agents of unstated purity (Table 3-3). Some of the subjects also were treated with protective or reactivating agents (case file data of Edgewood subjects, Appendix E).

For this review, approximately 15% (219) of the medical records were selected on the basis of high dosage, repetitive exposure, or the presence of additional physiologic stress. Case records were also selected randomly on the terminal digit of the case number (i.e., ending in 3). Brief summaries were made available to the Panel. In addition, complete records of 32 persons given EA 3148 intravenously were examined. These were chosen because this agent is considered the most potent of the anti-ChEs tested (156). In all cases, subjects were identified by pharmacologic class, by agent, and by letter code; names were withheld.

The case summaries are brief and anecdotal. With the exception of one case, they deal only with the period immediately after the test dose. There are no reports of neurologic or psychologic examinations (with the exception of subject A5J), and only four reports of EEGs--three made before treatment with the anti-ChE agent, and one after. The medical records of subjects tested with EA 3148 comprise doctors' orders, observations of the patient recorded largely by nurses, a clinical master log recording only blood pressure and pulse before and after exposure, results of a test of numerical facility, and ChE concentrations in blood, plasma, and red cells, before and at intervals after exposure. Physician's workup, progress, and discharge notes are absent. Descriptions of the subjects' reactions are rather vague and are certainly not sufficient for careful analysis of long-term effects of these agents. The various subjective complaints listed in the case-file data of Appendix E are not further documented by examination of findings.

The major focus of this Panel's investigation is the possibility of long-term or delayed effects. Essentially, anecdotal information is provided in the case summaries and some of this information seems to suggest that immediate psychological effects can follow the administration of both reversible and irreversible cholinesterases. However, the summaries do not provide hard data that would allow the panel to address, in a definitive manner, the question of whether or not there is a possibility of long-term or delayed effect. As noted above, the case summaries were concerned with the period immediately after administration of the agent and therefore give no indication of the possibility of long-term adverse effects. The paucity of data in the medical records prevents further study in relation to the goal of this report. However, there are published papers which appear to demonstrate long lasting EEG changes from single or repeated exposure to OPs (83).

In reviewing the detailed case report of the single subject who experienced long-lasting psychologic symptoms, the Panel noted that he had had both physical and psychologic evaluations before acceptance in the volunteer program. Evidently, this included examinations and psychologic testing (the MMPI was mentioned). It is possible that these data, if available, can be used as a basis for comparison with a long-term followup study, if such is undertaken.

MORTALITY DATA

A preliminary review of the standardized mortality ratios (SMRs) suggests that mortality was not significantly increased by exposure to anti-ChE chemicals, as tested at Edgewood (Table 4). Whether one looks at subjects exposed to sarin only or sarin plus another chemical (sarin total), VX only or VX plus another chemical (VX total), anti-ChE only or anti-ChE plus another chemical, the SMRs are roughly 80% (or less) of the rates expected for the U.S. population as a whole. This presumably reflects the fact that those who enter the military service do not have chronic diseases. These findings provide a first approximation of mortality and were intended to reveal trends. A more thorough evaluation of mortality findings is contained in Chapter 4.

EVALUATION OF THE LIKELIHOOD OF LONG-TERM ADVERSE HEALTH EFFECTS

The following commentary is based on evaluation of the known adverse health effects of anti-ChEs on humans and animals, the type, number, amount, and route of administration of agents, and medical records of subjects released by the Army.

The subjects were experimentally given anti-ChE agents in the CW testing program during the 1950s and 1960s. The mortality data, collected during 1981, led to the conclusion that, during the elapsed time since testing, subjects were no more likely to have died than comparable soldiers outside the testing program. Morbidity data are unavailable and should be collected; special focus should be placed on the possible long-term adverse health effects highlighted below.

It must be emphasized that the following opinions represent essentially extrapolations from known data. Although there are published and nonpublished results indicating that EEG changes can occur for one year after exposure to organophosphorus drugs, there is no documentation available as to longer-lasting effects. The statements offered below therefore represent conjecture on the part of Panel members and their consultants, who collectively have broad experience with and expertise on anti-ChEs and their effects in humans and animals.

BRAIN DYSFUNCTION

There are no records to indicate that the soldiers might have experienced subtle changes in brain function that lasted for long periods after discharge from the test environment (except, perhaps, in the one case cited above). But examination of primates and humans exposed to single or repeated doses of sarin has revealed statistically significant changes in the EEG that are apparent for at least a year after exposure (83). These observations have yet to receive laboratory confirmation, and their exact importance is unknown. There are also a number of anecdotal reports of minor disorders of affect, sensation, memory, and sleep in humans accidentally exposed to OP war gases or insecticides. The underlying changes in brain structure and function are unknown. The EEG changes are compatible with abnormalities of sleep and behavior. It is also possible that pre-existing psychopathologic conditions were exacerbated. Some subjects were treated with soman, which has been alleged (in a single, limited experimental study) to induce in laboratory rats profound and irreversible neuropathologic changes in vitally important regions of the brain (128). This laboratory study has not been replicated, and the duration and lifetime significance, if any, of the above-noted effects in humans are not known; they should be looked for in both adult life and old age. There is no evidence from the medical records that subjects given potent anti-ChE chemicals (e.g., military nerve agents) experienced the more severe acute effects that were produced in animals with large doses of soman. In particular, there are no reports of respiratory insufficiency or convulsions that could precipitate periods of hypoxia and lead to permanent damage of brain tissue.

JUNCTIONAL NEUROMYOPATHY AND DELAYED NEUROPATHY

Medical records of the vast majority of subjects tested do not refer to induction of the muscle hyperactivity that is associated with acute neuromyopathy in laboratory animals and humans poisoned by OP insecticides. Although there was no systematic attempt to inspect tested subjects for fibrillation or fasciculation of skeletal musculature during or after testing, the doses of anti-ChEs used in relation to the route of application are considered unlikely to have induced muscle damage in most of the subjects. Even if a subacute neuromyopathy had occurred in a few subjects, especially those who reportedly displayed weakness and muscle twitching after exposure, experience with laboratory animals suggests that these changes would have been resolved within weeks.

Although some of the OP agents tested can induce peripheral neuropathy in laboratory animals, the doses needed to induce clinical signs greatly exceed the LD_{50} for that species (105, 112, 113). Humans are generally understood to develop neuropathy after receiving doses comparable with those which induce clinical signs in hens (69, 121); because the doses used on the test subjects were far below those needed to induce experimental neuropathy, it is most unlikely that any subject experienced delayed onset of distal limb paralysis. However, clinical signs of neuropathy in experimental animals occur some time after the onset of CNS-PNS distal axonopathy, after a particular number of nerve fibers have undergone degeneration. It is therefore not possible to rule out the chance that subclinical pathologic changes occurred in vulnerable nerve-fiber pathways in subjects treated with agents known to be capable of inducing neuropathy. PNS nerve fibers probably undergo regeneration and re-establishment of end-organ connection promptly after the degenerative phase has terminated; damaged CNS fibers are unlikely to regenerate functional connections, but minor damage of this type usually does not induce noticeable functional impairment. Development in the test subjects of anything more than minor and transient sensorimotor manifestations as an expression of such putative damage is considered most unlikely and no such symptoms were recorded. Neurologic examination would reveal signs of long-term CNS changes, such as the Babinski sign (extensor plantar response), hyperactive ankle or knee jerk, and spastic or ataxic gait. Such signs would be permanent attributes of a person who suffered this type of CNS damage, but such changes in the test subjects are considered unlikely.

OTHER ADVERSE HEALTH EFFECTS

Mutagenicity, Carcinogenicity, and Male Reproductive Effects

There is little information on mutagenicity, carcinogenicity, and male reproductive effects, in relation to anti-ChE chemicals. Experimental evidence suggests that malathion does not pose a mutagenic or carcinogenic risk to exposed humans, nevertheless, some scientists disagree with this conclusion (157). The safety of the other compounds could not be confidently determined, because of the absence of laboratory studies. Nevertheless, the Panel is unaware of any reports linking these adverse health effects to single or repeated exposure to anti-ChE agents. Furthermore, there is no suggestion of increased mortality from carcinogenicity, although final judgment on this issue must be reserved until mortality and morbidity data are collected and analyzed.

Information on whether the tested subjects have sired children and on the state of health of their offspring since testing would be helpful in evaluating the possibility of anti-ChE-induced mutagenicity or adverse effects on the male reproductive system.

Cataractogenicity

On the basis of a review of the literature, it appears highly unlikely that single or occasional systemic exposure of young adult subjects to anti-ChE agents would result in the development of initially undetectable, long-term damage to the eye.

Blood Changes

Abnormalities of blood coagulation reported in persons exposed to particular anti-ChEs are considered reversible. Therefore, no long-term adverse effects on hemocoagulation in the test subjects are foreseen.

CONCLUSIONS

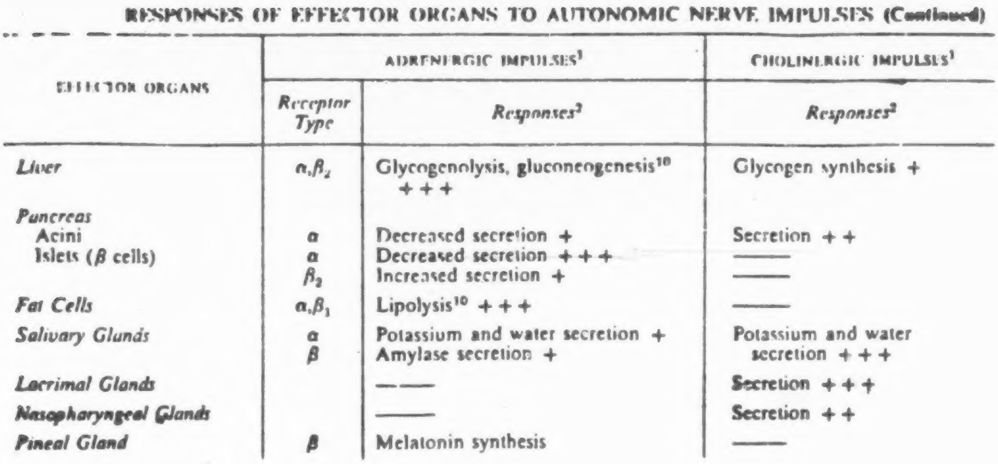
The panel concludes that although no evidence has been developed (to date) that any of the anticholinesterase test compounds surveyed carries long-range adverse human health effects in the doses used, the results of an ongoing NAS/NRC morbidity study may shed further light on this issue. The panel therefore is unable to rule out the possibility that some anti-ChE agents produced long-term adverse health effects in some individuals. Exposures to low doses of OP compounds have been reported (but not confirmed) to produce subtle changes in EEG, sleep pattern, and behavior that persist for at least a year. Whether the subjects at Edgewood incurred these changes and to what extent they might now show these effects are not known. If such changes occurred and persisted, they would be difficult to detect now. They could be determined scientifically only by a new study in which EEG, sleep state, and psychologic-test scores were compared with those from nonexposed control subjects. This might be considered, if reasonable suspicion develops, based on responses obtained in the referenced morbidity study, that selected subjects experienced behavioral changes traceable in onset to experimental exposure to the anti-ChE agents.

TABLE 1

RESPONSES OF EFFECTOR ORGANS TO AUTONOMIC NERVE IMPULSES

EFFECTOR ORGANS	ADRENERGIC IMPULSES[1]		CHOLINERGIC IMPULSES[1]
	Receptor Type	*Responses*[2]	*Responses*[2]
Eye			
Radial muscle, iris	α	Contraction (mydriasis) + +	———
Sphincter muscle, iris		———	Contraction (miosis) + + +
Ciliary muscle	β	Relaxation for far vision +	Contraction for near vision + + +
Heart			
S-A node	β_1	Increase in heart rate + +	Decrease in heart rate; vagal arrest + + +
Atria	β_1	Increase in contractility and conduction velocity + +	Decrease in contractility, and (usually) increase in conduction velocity + +
A-V node	β_1	Increase in automaticity and conduction velocity + +	Decrease in conduction velocity; A-V block + + +
His-Purkinje system	β_1	Increase in automaticity and conduction velocity + + +	Little effect
Ventricles	β_1	Increase in contractility, conduction velocity, automaticity, and rate of idioventricular pacemakers + + +	Slight decrease in contractility claimed by some
Arterioles			
Coronary	α,β_2	Constriction +; dilatation[3] + +	Dilatation ±
Skin and mucosa	α	Constriction + + +	Dilatation[4]
Skeletal muscle	α,β_2	Constriction + +; dilatation[3,5] + +	Dilatation[6] +
Cerebral	α	Constriction (slight)	Dilatation[4]
Pulmonary	α,β_2	Constriction +; dilatation[3]	Dilatation[4]
Abdominal viscera; renal	α,β_2	Constriction + + +; dilatation[5] +	———
Salivary glands	α	Constriction + + +	Dilatation + +
Veins (Systemic)	α,β_2	Constriction + +; dilatation + +	———
Lung			
Bronchial muscle	β_2	Relaxation +	Contraction + +
Bronchial glands	?	Inhibition (?)	Stimulation + + +
Stomach			
Motility and tone	α_2,β_2	Decrease (usually)[7] +	Increase + + +
Sphincters	α	Contraction (usually) +	Relaxation (usually) +
Secretion		Inhibition (?)	Stimulation + + +
Intestine			
Motility and tone	α_2,β_2	Decrease[7] +	Increase + + +
Sphincters	α	Contraction (usually) +	Relaxation (usually) +
Secretion		Inhibition (?)	Stimulation + +
Gallbladder and Ducts		Relaxation +	Contraction +
Kidney	β_2	Renin secretion + +	———
Urinary Bladder			
Detrusor	β	Relaxation (usually) +	Contraction + + +
Trigone and sphincter	α	Contraction + +	Relaxation + +
Ureter			
Motility and tone	α	Increase (usually)	Increase (?)
Uterus	α,β_2	Pregnant: contraction (α); nonpregnant: relaxation (β)	Variable[8]
Sex Organs, Male	α	Ejaculation + + +	Erection + + +
Skin			
Pilomotor muscles	α	Contraction + +	———
Sweat glands	α	Localized secretion[9] +	Generalized secretion + + +
Spleen Capsule	α,β_2	Contraction + + +; relaxation +	———
Adrenal Medulla		———	Secretion of epinephrine and norepinephrine

TABLE 1 (contd)



RESPONSES OF EFFECTOR ORGANS TO AUTONOMIC NERVE IMPULSES (Continued)

EFFECTOR ORGANS	ADRENERGIC IMPULSES[1]		CHOLINERGIC IMPULSES[1]
	Receptor Type	*Responses*[2]	*Responses*[2]
Liver	α,β_2	Glycogenolysis, gluconeogenesis[10] + + +	Glycogen synthesis +
Pancreas			
Acini	α	Decreased secretion +	Secretion + +
Islets (β cells)	α	Decreased secretion + + +	——
	β_2	Increased secretion +	——
Fat Cells	α,β_1	Lipolysis[10] + + +	——
Salivary Glands	α	Potassium and water secretion +	Potassium and water secretion + + +
	β	Amylase secretion +	
Lacrimal Glands		——	Secretion + + +
Nasopharyngeal Glands		——	Secretion + +
Pineal Gland	β	Melatonin synthesis	——

Koelle, G.B. In: The Pharmacological Basis of Therapeutics, Goodman, L.S. and Gilman, A, eds.), 5th edition. Macmillan Publishing Co., New York, 1975. Pg. 408.

[1] Responses are designated 1 + 10^3 + 10 provide an approximate indication of the importance of adrenergic and cholinergic nerve activity in the control of the various organs and functions listed.

[2] Dilatation predominates in situ due to metabolic autoregulatory phenomena.

[3] Cholinergic vasodilatation at these sites is of questionable physiological significance.

[4] Over the usual concentration range of physiologically released, circulating epinephrine, B-receptor response (vasodilation) predominates in blood vessels of skeletal muscle and liver; a-receptor (vasoconstriction), in blood vessels of other abdominal viscera. The renal and mesenteric vessels also contain specific dopaminergic receptors, activation of which causes dilatation, but their physiological significance has not been established.

[5] Sympathetic cholinergic system causes vasodilatation in skeletal muscle, but this is not involved in most physiological responses.

[6] It has been proposed that adrenergic fibers terminate at inhibitory B receptors on smooth muscle fibers, and at inhibitory a receptors on parasympathetic cholinergic (excitatory) ganglion cells of Auerbach's plexus.

[7] Depends on stage of menstrual cycle, amount of circulating estrogen and progesterone, and other factors.

[8] Palms of hands and some other sites ("adrenergic sweating").

[9] There is significant variation among species in the type of receptor that mediates certain metabolic responses.

TABLE 2

Effects of Cholinergics in Normal and Psychotic Persons*

Drug	Effect on Normal Person	Effect on Psychotic Person
Physostigmine antichEs	Depression; Psychomotor retardation	Improvement, particularly of thought disorder; No effect; antagonism of methylphenidate activation
Organophosphorus antichEs	Dysphoria; nightmares; excessive dreaming; hallucinations and delusions; schizoid reactions; auditory hallucinations; paranoid and religious delusions; agression	Some improvement in hebrephrenics; exacerbation in most cases (paranoids)
Choline	Depression	Improvement
Arecoline, oxotremorine		Increased interaction, lucid interval

*Derived from review papers by Singh and Lal (79) and by Karczmar and Richardson (80) and papers of Gershon and Shaw (81), Metcalf and Holmes (82), Duffy and Burchfiel (83), and Karczmar and Ohta (84).

TABLE 3

Summary of Tests Conducted and Records Selected for Anticholinesterase Chemicals

Tox No.	Compound	No. Subjects Tested	No. Records Selected
A-1[b]	Sarin, GB, 1208	246	25
A-2[b]	VX, 1701	740	75
A-3[b]	GA, tabun	26	13
A-4[b]	GF, 1212	21	10
A-5[b]	GD, 1210, soman	83	10
A-6[b]	DFP, diisopropylfluorophosphate	11	5
A-7[b]	EA 3148	32	16
A-9[c]	THA	15	5
A-10[d]	Eserine, physostigmine	138	23
A-11[d]	Prostigmine, neostigmine	22	5
A-12[e]	Hexafluorenium (Mylaxen)	11	11
A-13[d]	Pyridostigmine	27	8
A-14[b]	Malathion	10	5
A-20[f]	Methacholine	9	3
A-21[f]	Urecholine	15	5
	Total	1,406	219

[a] Two sets of records, one based on high dose, another based on random selection; each set contains 219 records.
[b] Organophosphate ester.
[c] Acridine.
[d] Carbamate.
[e] Quaternary ammonium inhibitor.
[f] Cholinergic agonist.

Table 4
Chemical Classification by Cause of Death, Anticholinesterase Chemicals

Causes of Death	Anticholinesterase Only			Anticholinesterase Total			Sarin Only			Sarin Total			VX Only			VX Total			Total All Classifications		
	O	E	O/E	O	E	O/E	O	E	O/E	O	E	O/E	O	E	O/E	O	E	O/E	O	E	O/E
All Trauma	7	10.01	.70	14	19.50	.72	1	2.55	.39	2	3.93	.51	5	6.02	.83	9	12.51	.72	66	94.89	.70
All Disease	12	13.80	.87	17	24.59	.69	5	4.69	1.07	6	7.33	.82	7	7.54	.93	10	14.91	.67	90	107.00	.84
Malignant Neoplasms	6	3.07	1.95	7	5.46	1.28	2	1.05	1.91	2	1.65	1.21	4	1.67	2.40	5	3.29	1.52	26	23.91	1.09
Cardiovascular System	4	5.65	.71	7	9.84	.71	2	2.04	.98	2	3.21	.62	2	3.00	.67	4	5.84	.69	34	42.03	.81
Cirrhosis of the Liver	1	1.03	.97	1	1.85	.54	1	.34	2.94	1	.53	1.89	0	.57	-	0	1.15	-	7	7.73	.91
Total All Causes*	26	32.58	.80	45	64.01	.70	9	10.87	.83	12	16.89	.71	16	18.07	.89	24	34.63	.69	218	268.38	.81
Total Subjects	602			1317			152			239			362			729			6548		

*This total includes the causes of death listed in this table plus all other causes, including cause not yet determined.

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3

ANTICHOLINERGICS

ATROPINE, SCOPOLAMINE, AND RELATED THERAPEUTIC ANTICHOLINERGIC CHEMICALS*

SITES OF ACTION

Anticholinergic drugs inhibit the actions of acetylcholine (ACh) on autonomic effectors that are innervated by postganglionic cholinergic nerves and on smooth muscles that lack cholinergic innervation; that is, they antagonize the muscarinic actions of ACh. They are therefore known as antimuscarinic agents or muscarinic cholinergic blocking agents. Because the main actions of all members of this class of drugs are qualitatively similar to those of the best-known member, atropine, the terms "atropinic" and "atropine-like" are also appropriately used.

In general, antimuscarinic agents have little effect on the actions of ACh at nicotinic receptor sites. Thus, at autonomic ganglia, where transmission normally involves an action of ACh on nicotinic receptors, atropine produces partial block only at relatively high doses. At neuromuscular junctions, where the receptors are nicotinic, extremely high doses of atropine or related drugs are required to cause any degree of blockade. However, quaternary ammonium analogues of atropine and related drugs generally have some degree of nicotinic blocking activity and consequently are more likely to interfere with ganglionic or neuromuscular transmission in doses only slightly greater than those that produce muscarinic block.

In the central nervous system (CNS), cholinergic transmission appears to be predominantly nicotinic in the spinal cord and both muscarinic and nicotinic at subcortical and cortical levels in the brain (2). Accordingly, many or most of the CNS effects of atropine-like drugs at ordinary doses are probably attributable to their central anticholinergic actions. At high or toxic doses, the central effects of atropine and related drugs consist, in general, of stimulation followed by depression; these are probably due to a combination of antimuscarinic and other actions. There is an increased release and turnover of ACh in the CNS associated with the administration of antimuscarinic drugs; this may result in the activation of nicotinic receptors in the brain and contribute to the central effects of this class of drugs (3). Because quaternary compounds penetrate the blood-brain barrier only poorly, antimuscarinic drugs of this type show little in the way of central effects.

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*Most of the information in this section is from Chapter 7 of Goodman and Gilman (1).

PERIPHERAL EFFECTS

Parasympathetic neuroeffector junctions in different organs are not equally sensitive to antimuscarinic agents. However, the relative sensitivity of various parasympathetically innervated organs to blockade by atropinic agents varies little among the drugs. Small doses inhibit salivary and bronchial secretion and sweating. Larger doses cause the pupils to dilate, inhibit accommodation of the eyes, and block vagal effects on the heart, so that the heart rate is increased. Still larger doses inhibit the parasympathetic control of the urinary bladder and gastrointestinal tract, thus inhibiting micturition and decreasing intestinal tone and motility. Even larger doses are required to inhibit gastric secretion and motility. Because only the primary phase of gastric secretion is controlled by the vagus, the remaining hormonally controlled secretion is unaffected. Thus, doses of any antimuscarinic drug that reduce the tone and motility of the stomach and the duodenum and inhibit gastric secretion also invariably affect salivary secretion, ocular accommodation, and micturition. The drugs produce reversibly the functional equivalent of resection or paralysis of postganglionic cholinergic nerves.

The actions and effects of particular antimuscarinic agents usually differ only quantitatively from those of atropine, a belladonna alkaloid, which is considered in detail as the prototype of the group.

CENTRAL EFFECTS

Atropine stimulates the medulla and higher cerebral centers. In doses used clinically (0.5-1.0 mg), this effect is usually confined to mild vagal excitation. The rate and occasionally the depth of breathing are increased, but this effect is probably the result of bronchiolar dilatation and the consequent increase in physiologic "dead space." With toxic doses of atropine, central excitation becomes more prominent, leading to restlessness, irritability, disorientation, hallucinations, or delirium. With still larger doses, stimulation is followed by depression, coma, and medullary paralysis. The latter may be primarily responsible for a fatal outcome. Even moderate doses of atropine may depress some central motor mechanisms that control muscle tone and movement. This effect has been used to advantage in the management of the tremor and rigidity of Parkinsonism.

Scopolamine in therapeutic doses normally causes drowsiness, euphoria, amnesia, fatigue, and dreamless sleep with a reduction in rapid-eye-movement (REM) sleep. However, the same doses of scopolamine occasionally cause excitement, restlessness, hallucinations, or delirium, especially in the presence of severe pain.

Doses of atropine required to inhibit peripheral responses to choline esters or anticholinesterase (anti-ChE) agents produce almost no detectable central effects. This may reflect difficulty of penetration of the drug into the CNS. In animals, atropine antagonizes the action of ACh applied locally to the cerebral cortex and spinal cord. However, atropine also depresses the effects of noncholinergic stimuli, indicating that the drug has central actions other than blocking cholinergic synapses.

The rise in body temperature due to the belladonna alkaloids is usually significant only after large doses. Nevertheless, in infants and small children, moderate doses induce "atropine fever." In atropine poisoning in infants, the temperature may reach 43°C or higher. Suppression of sweating is doubtless an important factor in the production of the fever, especially when the environmental temperature is high, but other mechanisms also may be important when large doses are taken. It has been suggested that atropine may exert a central effect on temperature regulation; however, animals that do not sweat, such as dogs, do not exhibit fever after atropine.

POISONING BY BELLADONNA ALKALOIDS

The deliberate or accidental ingestion of belladonna alkaloids or other drugs with atropinic properties is a major cause of poisonings. Infants and young children are especially susceptible to the toxic effects of atropinic drugs (4). Delirium and toxic psychoses, without undue peripheral manifestations, have been reported in adults after instillation of atropine eyedrops.

Fatalities from intoxication with atropine and scopolamine are rare, but they sometimes occur in children, in whom 10 mg or less may be lethal. Idiosyncratic reactions are more common with scopolamine than with atropine, and ordinary therapeutic doses sometimes cause alarming effects. Table 1 lists undesirable responses or symptoms of overdose associated with various doses of atropine.

SYMPTOMS AND SIGNS OF ATROPINE POISONING

Symptoms and signs of atropine poisoning develop promptly after ingestion. The mouth becomes dry and the person experiences a burning sensation; swallowing and talking are difficult or impossible; and there is marked thirst. The vision is blurred, and photophobia is prominent. The skin is hot, dry, and flushed. A rash may appear (more likely in children), especially over the face, neck, and upper part of the trunk; desquamation may follow. Body temperature rises, especially in infants. The pulse is weak and very rapid although tachycardia may be less pronounced in infants and old people. Palpitation is prominent, and blood pressure may be increased. Urinary urgency and difficulty in micturition are sometimes noted. Abdominal distention may develop, especially in infants.

The patient is restless, excited, and confused and exhibits weakness, giddiness, and muscular incoordination. Gait and speech are disturbed. Nausea and vomiting sometimes occur. The behavior and mental signs may suggest an acute organic psychosis. Memory is disturbed, orientation is faulty, hallucinations (especially visual) are common, the sensorium is clouded, and mania and delirium are not unusual (5). The diagnosis of an acute schizophrenic episode or alcoholic delirium has been mistakenly made, and some patients have been committed to psychiatric institutions for observation and diagnosis. The syndrome often lasts 48 h or longer and may be punctuated by convulsions. Depression and circulatory collapse occur only in cases of severe intoxication; blood pressure decreases, respiration becomes inadequate, and death due to respiratory failure follows after a period of paralysis and coma (6).

TREATMENT

When intoxicating doses of atropine have been taken orally, gastric lavage and other measures to limit intestinal absorption should be initiated without delay. Physostigmine, long overlooked as a possible antidote to atropine poisoning, is the rational therapy. For example, the slow intravenous injection of 1-4 mg of physostigmine (0.5-1.0 mg in children) rapidly abolishes the delirium and coma caused by large doses of atropine. Because physostigmine is metabolized rapidly, the patient may again lapse into coma within 1-2 h, and repeated doses may be needed (4,5,7).

MECHANISM OF ACTION

The major action of the antimuscarinic agents is a competitive antagonism of the actions of ACh and other muscarinic agonists. The antagonism can be overcome by increasing sufficiently the concentration of ACh at receptor sites of the effector organ. The receptors affected are those of peripheral structures that are either stimulated or inhibited by muscarine--that is, exocrine glands and smooth and cardiac muscle. Much evidence supports the notion that atropine and related compounds compete with muscarinic agonists for identical binding sites on muscarinic receptors (2,8,9).

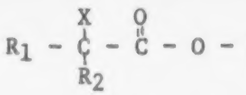
ABSORPTION, FATE, AND EXCRETION

The belladonna alkaloids are absorbed rapidly from the gastrointestinal tract. They also enter the circulation when applied locally to mucosal surfaces. Only limited absorption occurs from the intact skin. Atropine disappears rapidly from the blood and is distributed throughout the entire body. Most is excreted in the urine within the first 12 h, in part unchanged. Only about 1% of an oral dose of scopolamine is eliminated as such in the urine. Traces of atropine are found in various secretions, including milk. The total absorption of quaternary ammonium derivatives of the alkaloids after an oral dose is only about 10-25 percent (10,11); nevertheless, some of these compounds can cause mydriasis and cycloplegia if applied to the eye.

PHARMACOLOGICAL PROPERTIES OF SYNTHETIC ATROPINE DERIVATIVES

After reports in the late 1950s of the psychotomimetic effects of a number of basic esters of substituted glycolic acids related to scopolamine and atropine, several hundred compounds were synthesized at Edgewood and by its contractors and by academic and industrial researchers. These compounds were intended to be more active centrally than peripherally. Because quaternary salts of these anticholinergic compounds usually penetrate the CNS only with great difficulty, such derivatives were of little interest in the testing program.

The compounds prepared and tested in the Edgewood program are listed in Table 2, and their structures are shown in the master file (Appendix B). The basic structures of these compounds, which are substituted glycolates, are as follows:



$$R_1 - \overset{X}{\underset{R_2}{C}} - \overset{O}{\overset{\|}{C}} - O - R_3$$ (basic structure of glycolates),

where X = $-OH$, $-CH_2OH$, $-OCH_3$, $-Cl$, $-Br$, $-I$, $-O_2CCH_3$,

R_1 and R_2 = hydrogen, phenyl, substituted phenyl, alkyl, cycloalkyl, ethylenic, olefinic, thienyl, and

R_3 = acyclic aminoalkyl, cyclic aminoalkyl, bicyclic amino alkyl (e.g., tropine or scopine for atropine and scopolamine, respectively).

Table 3 lists the relative potencies (comparing central with peripheral effects) of many of these derivatives. Note that BZ and EA 3443 exhibit extensive central effects for a long period; the studies at Edgewood emphasized these chemicals, as does this report.

In a comparison of a series of structurally related anticholinergic compounds, there was less discrepancy in potency with respect to peripheral than central actions. Because data on their effects on man are limited, most of the information was derived from animal behavioral studies, which in general predict psychotomimetic actions in man.

BZ and other quinuclidinyl glycolates containing at least one phenyl and a cycloalkyl group in the acid moiety were the most potent and the longest-acting both in animals and in man. The corresponding piperidyl glycolates were one-fifth to one-half as active as the quinuclidinyl esters. Atropine is equipotent (although of shorter duration) to BZ in producing peripheral anticholinergic effects, but its central actions are much less pronounced.

ROLE OF DRUG DISPOSITION IN EXPLAINING DIFFERENCES AMONG ANTICHOLINERGIC COMPOUNDS

ATROPINE

Numerous reports dealing with the disposition of labeled atropine in animals and are reviewed by Wills in Appendix I. Investigators have been only partially successful in their efforts to compare the pharmacologic actions and relative potencies of the various anticholinergic compounds in the CNS with their distribution in brain or their binding to brain components. For instance, BZ has been reported to adsorb to isolated brain mitochondria about 3 times as avidly as atropine (12). The affinity of BZ for peripheral muscarinic receptors was also greater than that of atropine (8), and atropine competed with BZ for specific binding sites in the brain. Because BZ is known to be more potent than atropine and its duration of action is appreciably longer (13), these drug effects could be due to differences in the affinities of the two drugs for specific receptor sites.

BZ

Snyder's group (14,15) used autoradiography to determine precisely the location of binding of $[^3H]$BZ in rat brain. In the cerebral cortex, the greatest binding of the benzilate was in the occipital cortex and the cortex of the cingulate gyrus, with decreasing binding in the cortex in the piriform area and the frontal pole. In the hippocampus, three strata of the archipallium were found to have approximately equal abilities to bind BZ, whereas the white matter of the alveus bound less. The high binding of BZ to the striate body was particularly striking in autoradiographs because of the comparatively low binding activity of the adjoining globus pallidus. Thalamic and hypothalamic nuclei exhibited less binding of BZ, as did the inferior and superior colliculi. Still lower in the binding of this ligand were cerebellar cortex, medulla oblongata-pons, and the cerebral peduncles. Nerve tracts--e.g., the optic chiasma, the cervical spinal cord, and the corona radiata--exhibited the lowest degree of binding.

The areas of the brain that retained the greatest concentrations of the label after intravenous injection of [^{3}H]BZ into cats (16) were motor cortex, sensory cortex, caudate nucleus, lateral geniculate, and medial geniculate. Smaller concentrations were retained in thalamus, hippocampus, hypothalamus, medulla oblongata, colliculi, cerebellar cortex, the pyramids of the medulla, cerebral white matter, and cerebellar white matter.

Both cholinomimetic and anticholinergic compounds reduced the retention of the label after [^{3}H]BZ treatment in some areas of the brain. The cholinomimetic compounds used were 1,2,3,4-tetrahydro-5-aminoacridine (THA) and S-diethylaminoethyl-diethylphosphorothionate, both inhibitors of cholinesterases. For the anticholinergic compounds, displacement of the label probably was related to the substitution of one drug by another.

Zvirblis and Kondritzer (12) found that a mitochondrial fraction prepared from rat brain adsorbed 3-4 times as much BZ as atropine in the physiologic range of pH. The optimal pH for adsorption of BZ was about 8.0, whereas that for atropine was about 9.7; however, at their own optimal pH values, the adsorptions of the two anticholinergic compounds were about the same. THA, Ca^{2+}, and $PO_4{}^{2-}$ decreased the adsorption of the benzilate by mitochondria.

Yamamura et al. (17) studied the adsorption of [^{3}H]BZ on the particulate fraction of homogenates of various regions of the brain of monkey after incubation with [^{3}H]BZ in phosphate buffer at a pH of 7.4. The regions that were the most avid adsorbers of the benzilate, in order of decreasing uptake, were putamen, caudate nucleus, occipital cortex, cingulate gyrus, postcentral gyrus, pyriform cortex, frontal cortex, superior colliculi, thalamus, and inferior colliculi. This distribution of adsorptive sites among various parts of the monkey brain incubated in vitro differs from that for intravenously injected [^{3}H]BZ in the cat (16). The two studies do agree that the caudate nucleus is one of the highest areas of localization of [^{3}H]BZ. Yamamura et al. (17) found that uptake of the label by various areas of brain was correlated with choline uptake and with choline acetyltransferase activity.

Interruption of the septal hippocampal tract and elimination thereby of cholinergic afferents to the hippocampus reduced by about 70% the activity of choline acetyltransferase in homogenates of the hippocampus, but did not alter the binding of [^{3}H]BZ by particles of such homogenates (8). The investigators proposed that presynaptic muscarinic sites that are innervated by the septal hippocampal nerve fibers do not bind the labeled benzilate, whereas postsynaptic muscarinic sites do bind it.

Ketchum (18) provided information that suggests that the brain is the only mouse organ that contains ^3H 48 h after intraperitoneal injection of $[^3H]BZ$. Perfusion of $[^3H]BZ$ through rat liver had indicated that this organ destroyed the compound quickly; 90% of the compound present originally in the perfusing fluid disappeared from the perfusate within 30 min, and less than 0.2% of the unaltered compound was found in the bile. The identity of the products was not determined.

The effects of BZ and Ditran, compared with atropine and scopolamine, have been examined to elucidate the mechanisms by which these compounds produce their effects. All these compounds have an extremely high affinity for muscarinic receptor sites, so the higher potency and longer duration of action of BZ must be related to other properties.

The long-lasting effects (up to 7 d) of a single injection of BZ in cats (19) raised the issue of possible irreversible interaction of BZ with brain sites. With another anticholinergic drug, $[^3H]$pyrrolidylmethylphenylcyclopentylglycolate (20), a small amount of radioactivity was still measurable 3 d after a single injection. Considering the high affinity of BZ for muscarinic receptors ($K_d = 10^{-11}$ M), it would not be surprising to find BZ still attached to brain receptors 5-7 d later, especially because pharmacologic effects of BZ are evident in man after 7-8 d. The prolonged action of BZ, compared with its somewhat shorter duration of binding to CNS structures, is not yet readily explainable.

ANIMAL TOXICOLOGY OF ANTICHOLINERGIC COMPOUNDS

IMMEDIATE EFFECTS

BZ

BZ has been chosen as the paradigm of the various anticholinergic test compounds used at Edgewood. It was used in a large number of volunteers and is probably one of the best documented. Because it is also one of the longest-acting of all the drugs, it is also more likely to cause persistent adverse effects. It was one of the first glycolates used in relatively high doses in man, so more years have elapsed in which long-range consequences might have occurred.

The pharmacology and toxicology of BZ have been studied extensively in animals. Much of the information was derived through a contract with Hazleton Laboratories, Falls Church. A summary of the preclinical pharmacology and toxicology in rats, dogs and monkeys is presented here; a list of other toxicity studies is given in Appendix F. Additional background information is contained in Appendixes G, H, I, J, K and L.

Rats. Repeated intravenous administration of BZ to male albino rats was carried out for 20 daily injections during a 4-wk period. The doses included 0.01, 0.1, and 1.0 mg/kg.

The most significant pharmacologic sign noted was mydriasis. The daily pattern of mydriasis noted after each injection suggested metabolic inactivation of BZ by rats within 24 h. No tolerance to the mydriatic response was, however, apparent during the course of the study. At the high dose there appeared to be a progressive diminution in the intensity of pharmacotoxic signs (other than mydriasis); that suggested development of tolerance or decreased responsiveness to BZ.

Other measured indicators of pharmacologic effect included body weight, food consumption, hematology, gross and microscopic pathologic signs and organ weights. No significant effect attributable to the repeated administration of BZ was found.

There were no indications of liver disease or malfunction directly attributable to BZ in chronic-toxicity studies. In some rats, there were a few small foci of periportal lymphocytic infiltration; but these occurred as often in saline-treated animals.

Dogs. Repeated intravenous administration of BZ to male and female mongrel dogs was carried out for 20 d over a 4-wk period. The dosages were 0.01, 0.1, and 1.0 mg/kg-d. The indicators of effect were gross pharmacotoxic signs, daily body weight, hematology, biochemistry, urinalysis, organ weights, organ-to-body weight ratios, and gross and microscopic pathology.

A minimal number of effects was produced by low dosages between the eleventh and thirteenth injection days. Both the intermediate and high dosages produced mydriasis, ptosis, decreased activity, ataxia, and weakness of limbs. Other signs were less frequently observed. An increase in sedimentation rate occurred at 4 wk in one low- and one high-dosage animal. Liver weight was high in one male from each test group. Testicular atrophy was observed in one male each from the intermediate- and high-dosage groups. A dose of 10 mg/kg induced bradycardia in all of five dogs and resulted in death due to cardiac arrest in two of the animals (21). Dogs given daily intravenous doses of 100 µg/kg for 14 consecutive days had the same LD_{50} as dogs that had not been so exposed, but the interval between injection of the daily dose and the appearance of ataxia increased from 4 min to 14 min during the period of pretreatment. In dogs trained in a conditioned-escape routine and then given graded intravenous doses of BZ, doses up to and including 12.5 µg/kg had no effect on performance. However, a dose of 25 µg/kg resulted in failure to escape by four of four dogs tested (22). None of the experiments summarized in this report demonstrated any persistent effect of exposure to BZ in surviving dogs.

Monkeys. Repeated intravenous administration of BZ at 0.01, 0.1, and 1.0 mg/kg-d to male and female monkeys was carried out for 20 d over a 4-wk period. The indicators of effect were gross pharmacotoxic signs, daily body weight, hematology, biochemistry, organ weights, organ-to-body weight ratios, and gross and microscopic pathology.

The most frequently observed signs were mydriasis at all dosages and decreased activity and ataxia at the two highest dosages. Blood sugar of one monkey given an intermediate dosage and serum transaminase of one monkey given a high dosage were increased at 4 and 2 wk, respectively. The testicular weight and organ-to-body weight ratio were high for one intermediate-dosage animal. All other findings were within control limits.

Cycloplegia persisted in monkeys for more than 7 d after exposure to BZ, whereas most other effects had disappeared after 2 d (18).

EA 3443

Hazleton Laboratories conducted preclinical toxicity studies with EA 3580, EA 3392, and EA 3443. The following information was abstracted for EA 3443.

A total of 20 daily intravenous injections of EA 3443 in male and female monkeys at 0.01, 0.1, and 1.0 mg/kg produced no hematologic or biochemical abnormalities. Organs examined microscopically presented no significant alterations (23).

A total of 20 daily intravenous injections of EA 3443 in mongrel dogs (two dogs per dose) at 0.01, 0.1, and 1.0 mg/kg produced no hematologic, urinary, or biochemical changes. Histopathologic findings were essentially normal (24).

EA 3834

Prompted by a report of hematuria in a subject receiving EA 3834, research was begun on the effects of these drugs on ureteral and bladder motility (25). Microscopic hematuria was shown to occur within several minutes after intravenous administration of EA 3834 and usually cleared within 1 h. The dogs had ureteral or bladder catheters in place and often had a few red blood cells in the urine before administration of the agent. However, the red cells became much more numerous after administration. No additional information is available.

The hematuria was most likely the result of a reduction in renal blood flow. It is not clear that the hematuria was produced by EA 3834.

LONG-TERM AND DELAYED EFFECTS

Atropine

Atropine sulfate was administered intramuscularly to rabbits daily for 100 d. At a dose estimated to be 5% of the LD_{50}, signs of toxicity were seen. Pathologic signs included weight loss, edema of most organs, hepatitis, pulmonary thrombosis, inhibition of spermatogenesis, thymic atrophy, and toxic changes in the gall bladder, spleen, and pancreas. The rabbits' survival of daily administration of atropine beyond the LD_{50} was related to their ability to maintain food intake (26).

Clidinium (Quartzan)

Clidinium was administered intragastrically to dogs at 1,5 and 25 mg/kg, 5 d/wk for 52 wk (27). The animals were observed for survival, behavior, and general physical condition. Blood, liver and kidney function were evaluated. Organs were examined microscopically at the conclusion of tests. Toxic signs of anticholinergic exposure were not seen. Hematologic characteristics were not significantly altered by these prolonged exposures. Clinical-chemistry measures and results of gross and microscopic evaluations of internal organs were within normal ranges.

Administration of clidinium for 1 yr at 5.0, 25, and 50 mg/kg in the diet of rats did not result in drug-related toxicity. Blood counts, clinical-chemistry measures, and results of gross and microscopic studies remained within normal limits (28).

Other Data

No study of the long-term administration of BZ to animals has been uncovered. The only long-term toxicity study with an ester of benzylic acid was with the ester with diethylaminoethanol; no significant pathemas were found in rats during this lifetime feeding study, but the lifetimes of the rats fed the benzilate may have been shortened somewhat. Feeding of the quaternized ester of 3-quinuclidinol to rats for a year (27) and gavaging of dogs with this compound 5 d/wk for a year (29) produced no significant pathemas.

With respect to genotoxicity, BZ has been found to have weak mutagenic activity in yeast cells in culture (30) and to produce gaps and breaks in chromosomes of bone marrow cells; it has not been proved to produce a heritable change in mammalian species. Diethylaminoethylbenzilate was more toxic cytogenetically than BZ, despite its lower anticholinergic activity. Unfortunately, there is too little information on these compounds to assume or exclude genotoxic effects. Although various test systems have been used (point mutations, chromosomal observations, and dominant-lethal effects), most observed effects probably resulted from general drug toxicity, rather than genotoxicity. The drugs have been tested at only a few concentrations, and interpretation of existing data does not permit estimation of the genotoxicity of BZ or even of atropine or scopolamine.

HUMAN TOXICOLOGY OF ANTICHOLINERGIC COMPOUNDS

DOSING CONSIDERATIONS

Atropine

Textbooks generally suggest that death from atropine poisoning may be expected after ingestion of doses above 100 mg. However, a review of the literature for the last 100 yr yielded a probit estimate of about 450 mg/person, based on several hundred cases of accidental poisoning, including some 40 fatalities. Because the oral route is somewhat less effective than the intravenous or intramuscular (and probably the inhalation) route, a conservative figure of 75 mg/person (approximately 900 μg/kg) is a working estimate for the human LD_{50}.

Other Anticholinergic Compounds

Acute toxicity data on the 21 glycolates studied in man in a cool climate show a fairly consistent ratio between the lethal dose in animals and the heart-rate-increasing dose in man. Extrapolation of the animal data therefore allows an estimate of the risk associated with human exposure. Deaths have occurred in seemingly healthy people when atropine has been given at doses only 50 times greater than the heart-rate-increasing dose. Thus, the estimated LD_{50} in man for BZ is about 200 μg/kg, whereas that for EA 3834 is about 60 μg/kg. This represents safety margins of about 35 for BZ and 100 for EA 3834, where safety margin is the ratio between LD_{50} and ID_{50}.

In a warm climate, these numbers need correction, because the heat loss from sweating is diminished. It is speculated that fatal hyperthermia or heatstroke could occur at doses very close to the ID_{50} for BZ and not more than 2 or 3 times the ID_{50} for EA 3834.

In the Edgewood tests, no volunteer received BZ at more than 10% of the LD_{50}. Probably no more than 30 or 40 subjects received more than 3% of the LD_{50}.

Few subjects received more than one dose of a glycolate. In the case of BZ, seven subjects were given the ID_{50} on two occasions, 2-3 wk apart. Two subjects received 8.0 µg/kg twice each, in a crossover double-blind study of the efficacy of physostigmine as an antidote. Eight subjects were given 1 µg/kg daily for 7 d (with no appreciable effects), and four received 2 µg/kg on three consecutive days (with cumulative effects approaching incapacitation on the third day.) Thus, fewer than 10% of all subjects received more than a single dose. The figures for other glycolates are lower. The total exposure of any person to glycolate never exceeded 3 times the ID_{50} dose in the entire series of tests.

USE OF ANTIDOTES

Anticholinergic agents are typically used as treatment for anticholinesterase poisoning and vice versa. Physostigmine, tetrahydroaminoacridine, and other cholinesterase inhibitors were used successfully as antidotes in several dozen subjects. The demonstration of physostigmine's effectiveness led to the first controlled study of its ability to reverse delirium due to scopolamine (31). Physostigmine has been used to overcome anticholinergic toxicity.

IMMEDIATE EFFECTS

Acute clinical findings were recorded by involved physicians and nurses when Edgewood volunteers underwent tests with 21 anticholinergic chemicals shown in Table 2-2. About 1,750 subjects were tested with agents in this pharmacologic class, 10% of which were summarized for review. Selection for inclusion in this summary was based on high dose, high frequency of administration, or additional physiologic stress; it was the intent to focus on the extreme cases. The summaries identify the compound administered, dose, route of exposure, and significant acute reactions or clinical findings.

Peripheral and Behavioral Effects

In the brain, the arousal system that maintains alertness during waking hours is depressed by the action of BZ; this leads to drowsiness, which may progress to stupor or even coma at higher doses. Simultaneously, the coordinating motor systems of the cerebellum seem to be affected, and that causes an unsteady gait and pronounced clumsiness. There is a subjective sensation of dizziness which may arise in part from disturbances of the vestibular systems in the brainstem. The modulation of muscle tone is affected, possibly owing to an action on the Renshaw interneurons in the spinal cord, and the results are an abnormal increase in tendon reflexes and a tendency toward stiffness and jerkiness in muscle movement. As the drug penetrates higher in the brainstem, hypothalamic systems show signs of disruption. There is a decrease in drinking and eating. Sleep may be inhibited. A tendency toward increased body temperature appears in a warm environment. Finally, the highest integrative systems of the brain are affected. Memory, attention, problem-solving, and judgment are all seriously impaired. The ability to distinguish reality from imagination is lost, and thoughts and words flow in an uncontrolled manner. For a moment, the subject may seem to be in contact with his surroundings, but within seconds he drifts rapidly into incoherent speech and disconnected action. He is, at this stage, truly incapacitated and cannot function sufficiently even to preserve his life. If not brought under control, the delirious subject can inadvertently become a casualty and may fall prey to a fatal accident.

During the early stage of intoxication, activity is reduced, movements are slow and ineffective, and the subject is generally unable to offer effective resistance and tends to be relatively tractable. Once this period (a few hours) has passed, his behavior becomes increasingly unpredictable, energetic, and dangerous.

In the features thus far described, BZ is a typical glycolate, indistinguishable qualitatively from atropine, scopolamine, EA 3834, EA 3443, EA 3580, EA 302,196, or any other compounds of the 21 in this family studied in volunteers.

EEG Effects

There is a close relationship between the changes in EEG patterns and the behavioral effects of drug administration. With high doses of atropine (10-30 mg), for example, or repeated administration, the EEG shows an increase in slow waves, a decrease in mean frequency, a decrease in the percent time and amplitude of alpha activity, and an increase in the fast frequencies, which can be seen to be 'riding' on the slow waves. There is a direct association between the EEG fast waves with behavioral restlessness and the EEG slow waves with stupor and cognitive defects. At ('toxic') doses, patients are in stupor or coma, with high heart rate and lowered blood pressure. The EEG demonstrates persistent high-voltage slow waves, with a minimum of alpha and fast frequencies.

EEG and behavioral effects were modified in a parallel manner by the concurrent administration of antagonistic drugs. For instance, when patients who exhibited a toxic delirium to Ditran or atropine were given tetrahydroaminoacridine, a cholinesterase inhibitor, the stupor was relieved and the EEG showed a decrease in both slow and fast frequencies.

During acute administration of various compounds, the time for recovery varied with dose with low doses, the peak effects of parenteral administration were seen in 0.5 h and lasted up to 6 h; with high doses, the effects persisted for up to 24 h. With toxic doses, a return to baseline occurred in the second day after administration. Followup EEG data are limited, the principal data being reports of atropine toxicity. In these studies, the few statements referring to EEG changes suggest that the effects disappear within a few days of the last exposure.

For the test compounds used at Edgewood, EEG data are available only for BZ, EA 3580, and EA 3834 on five, two, and one patients, respectively. The pretreatment and posttreatment EEG records appeared to be within normal limits and reflected effects observed for other CNS-active anticholinergic drugs. Neuropsychologic effects of the exposures to the agents were transient, and test values reverted to baseline. The EEG changes and neuropsychologic effects were temporally related, and a similar reversion to normal EEG patterns was anticipated. No evidence of persistence of behavioral or EEG effects after these experimental trials was reported by Klapper et al. (32).

BZ Case Reports

Data on the 36 subjects whose records were selected for evaluation from the records of 354 subjects treated with BZ indicate that BZ is active when it is given by the intravenous, intramuscular, aerosol inhalation, or oral route. The fragmentary data available indicate that the higher the dose the greater the effects and the longer their duration. Understanding of the timecourse of effects was confounded by erratic written documentation, which at best was rather sparse, and by the introduction, at various times after exposure to the drug, of treatment with cholinomimetics, such as physostigmine and tetrahydroaminoacridine. Both these treatments ameliorated, at least temporarily and partially, some of the effects of BZ.

Intravenous Injection. BZ doses of 4.8 and 8 µg/kg were used in four subjects. The time course was approximately as follows:

- 1 h: Dry mouth, flushed face, numbness in extremities, and sleepiness.
- 4 h: Above, plus twitching, yawning, clonus and abnormal reflexes, and ataxia.
- 24 h: Above, plus disorientation, hallucinations, and poor performance on psychometric tests.
- 36 h: Hallucinations persisting without treatment.
- 48 h: Diminished nonverbal and motor skills.

Single Intramuscular Doses. BZ doses of 5.0-6.4 µg/kg were used in 15 subjects. These subjects were treated at various times with the antidotes mentioned above. The time course of action of the drug by this route was similar to that by intravenous injection; in fact, more symptoms were reported with this route, but this may have been due to differences in the completeness of reporting. The time course was approximately as follows:

- 10 min: Light-headedness and giggling.
- 30 min: Dry mouth, blurred vision, nausea, chilly sensations, and twitching.
- 1 h: Flushed skin, incoordination, fatigue, unsteadiness, sleepiness, and quivering legs.
- 2 h: Many of the above, plus poor concentration, restlessness, hallucinations, slurred speech, and muscle fasciculations.
- 3 h: Above, plus tremors.
- 4 h: Above, plus difficulty in handling subject and belligerence; pulse, 136.

- 8 h: Above, plus delirium and hallucinations.
- 24 h: Persistent delirium, hallucinations, restlessness, unsteadiness, and increased pulse in some but not all subjects.
- 48 h: Persistent impairment of function in some and return to apparent normal state in others, depending on vigor of antidotal treatment.

Repeated Intramuscular Injection Six Times Over Same Number of Days. This program was used in four subjects. Reporting was extremely fragmentary, but perhaps not much happened, inasmuch as the doses used were quite small--0.5 µg/kg. A maculopapular rash that cleared when treatment was stopped was noted twice, and dysphagia was noted in another subject. No mydriasis was seen. No tolerance could be demonstrated, probably because the dose was below threshold. No cumulative effect was noted.

Aerosol. Doses used ranged from 5.3 to 17.1 µg/kg in eight subjects. Understanding of the clinical manifestations was confounded by the use of antidotes at various times after exposure to the drug. The time course was as follows:

- 30 min: Restlessness, sleepiness, nausea, dry mouth, weakness, and coldness of extremities.
- 1 h: Above, plus dizziness.
- 2 h: Above, plus flushed skin.
- 4 h: Above, plus unsteadiness and disorientation.
- 8 h: Above, plus hallucinations.
- 24 h: Residual confusion, disorientation, hallucinations, and delirium in some, but not all, subjects.
- 48 h: Residual mental impairment, jumpiness, and shakes in some and improvement in others, depending on amount of antidote used.

In one subject, fever and spastic movements of the head were noted; he was treated vigorously with antidotes. At 3 h, he became unresponsive, showed decerebrate rigidity, had a high heart rate, and had urinary retention; he was treated vigorously with antidotes. Five years later (1968), he was hospitalized for microscopic hematuria and was shown by renal biopsy to have focal glomerulitis.

Oral. Doses ranged from 4.5 to 5 µg/kg and were given to five subjects. The major focus seemed to be on following heart rates and the EEG. Clinical manifestations noted with the other routes of administration probably also occurred here. Some heart rates became fairly high (130/min). One subject had an EEG tracing that showed episodes of light sleep with spindling.

Summary. In spite of the sketchy records, BZ appears to be an anticholinergic hallucinogen quite comparable with JB-329 (Ditran) or others of the JB series or with scopolamine. The onset of effects is usually rapid, regardless of the route of administration, and the duration is often a function of the dose. Although pharmacokinetic data on these compounds in man are unavailable, there is some evidence of a dose-response relationship. Without treatment to counter the effects of these strong central anticholinergic agents, some effects may persist for several days. For instance, one subject who had received BZ displayed hyperthermia, tachycardia, and spastic movements for a few hours, and required vigorous treatment. He was discharged 6 d after exposure, well oriented and with normal appearance. No further information is available on him; the Veteran's Administration did not find his military records. Another subject developed signs of decerebrate rigidity with limb twitching. Two separate medical opinions were recorded: toxic encephalopathy and BZ delirium. The subject was discharged 6 d after exposure. EEG tracing 20 d after exposure was normal. His VA hospital record provided no useful information.

EA 3834

EA 3834 is somewhat distinctive from the other test compounds. The intravenous ID_{50} is approximately 5.7 µg/kg base equivalent, about the same as that for BZ. Although the time of onset of severe effects of EA 3834 is inversely related to dose (about 35 min for the ID_{50} and about 10 min for 3 times the ID_{50}), the duration of severe effects is roughly constant within this dose range, namely 6-9 h. The constancy of recovery time from EA 3834, which is rather unusual for these drugs, reduces the period of medical care required for higher-dose casualties. In contrast, doubling the ID_{50} prolongs BZ incapacitation about 40 h, as it does for EA 3443 and EA 3580.

Physostigmine has proved effective as an antidote to EA 3834, as it has with many other glycolates. Within 20 min of a single intramuscular injection of 1 mg of physostigmine salicylate per 18 g of body weight, the stuporous subject becomes alert and able to perform at close to his normal level.

Possible renal toxicity has been reported in two men after the use of this compound. One volunteer who had received the agent intravenously had red blood cells in his urine shortly after the completion of his test. Extensive workup failed to uncover any definite kidney disease or lesion to account for the bleeding, which persisted intermittently for a year after exposure. Such a phenomenon had not been noted in any previous glycolate study. It may be related to a pre-existing abnormality that did not show up in routine screening. No additional information is available on the second patient. Animal studies had suggested that such bleeding could indeed result from EA 3834, from BZ, and even from atropine. On careful review of the experiment, it was concluded that the bleeding could be ascribed to techniques used and animal selection. In man, the effect of atropine on renal function was investigated, and no abnormalities were observed. Testing of EA 3834 was cautiously resumed in human subjects, and renal function was monitored with frequent pretest and posttest urine examinations. In a total of 69 subjects, including those who participated in field tests, no additional cases of bleeding were encountered, even though the subjects received up to 3 times the dose administered to subjects with hematuria. A decrease in glomerular filtration was observed in some subjects, but this also occurred in a control population. Nevertheless, continued vigilance concerning possible renal effects seems warranted.

Overall Evaluation of Immediate Adverse Effects

In general, in all the testing programs there were few violent responses or injuries, largely because of surveillance and safety precautions taken at Edgewood. The only other laboratory abnormalities encountered in the Edgewood records involved an increase in liver enzyme values of two volunteers within about 2 wk of exposure to CAR 302,368. These abnormalities reversed after a few weeks, and it was unclear whether the changes resulted from the drug administration. Several cases of pyuria after exposure to EA 3580, or related drugs, and running of an obstacle course, were reported, but the drug etiology is unclear.

LONG-TERM AND DELAYED EFFECTS

Delayed effects of the acute cholinergic drugs were extremely rare. One person reported by Klapper *et al*. (32) experienced "flashbacks" while still at Edgewood Arsenal. Edgewood records showed that he received EA 3834 intravenously. Medical records on this subject obtained from the VA were voluminous, but did not disclose any significant findings. Six-month followup studies performed by Hart and Balter (33) on related glycolates failed to demonstrate significant cognitive changes. Volunteers in a prospective psychometric study who had received EA 3167 showed no evidence of residual drug effects.

Two nonvolunteers--one military (a chemist) and one civilian (a pharmacologist)--were accidentally exposed to unknown doses of EA 3167, the most persistent of the glycolates studied. In each case, subjective and objective observations of performance over a period of 6-12 mo indicated that mild, but nontrivial, impairment of cognitive function could be discerned for approximately 6 mo, after which seemingly full recovery ensued. These patients were of superior intellect and had occupations that required optimal cognitive function for them to be successful. It is possible that in less demanding assignments they might not have been aware of residual deficits. Similar persistent decrements were not reported by BZ volunteers.

An additional ancedotal report (L. G. Abood, personal communication, 1982) concerns an evaluation of the psychologic state and well-being of three subjects who had been exposed to BZ during the preceeding 10 yr. Subject A had an oral dose of approximately 5 mg during the summer of 1969; subject B, an oral dose of approximately 10 mg in June 1972; and subject C, an oral dose of 5-10 mg in October 1976. All three were graduate students who had surreptitiously undertaken self-experimentation and who had been engaged in laboratory experiments with the drug. All experienced hallucinations, delirium, confusion, amnesia, and the full spectrum of psychologic, neurologic, and autonomic responses associated with the drug. Within 2-4 h after ingestion, all three were hospitalized in the psychiatric ward at the University of Rochester, Strong Memorial Hospital and retained for various periods (3-6 d).

Regarding the possible long-range effects of the drug experience, all three subjects were having difficulties both adjusting to graduate education and in their personal lives immediately before taking BZ. Subjects A and C had entertained the possibility of either dropping out or transferring to another university, largely because of inadequate academic performance. Within a few months after the drug experience, all three demonstrated definite improvement in both academic performance and psychologic well-being. They all appeared to be more out-going, communicative, and enthusiastic about graduate school. This assessment was shared by a number of faculty members and fellow students. They all eventually received Ph.D. degrees with distinction. They are now pursuing an active, productive research and postgraduate education. They all appear well-adjusted and well-motivated and seem to be leading productive, self-fulfilling careers. None of the subjects has experienced any adverse sequelae that might be attributable to the drug experience.

In the absence of additional reports of long-range consequences in subjects who had received the anticholinergic test compounds, data have been sought on delayed adverse reactions to other anticholinergic drugs used therapeutically, such as Ditran and atropine.

Since the introduction in 1958 of Ditran for the treatment of depressed patients (34), the number of persons receiving the drug for depression and other psychiatric disorders has exceeded 2,000 (34-37). During 1957-1963, Ditran was used in at least 200 people, many of whom were private patients. The treatment involved the intramuscular administration of 10-30 mg of Ditran to a depressed patient--a dose that generally produces the full spectrum of behavioral and neurologic effects attributable to the centrally active anticholinergics. The patient's psychiatric condition was closely observed and evaluated for the next few months. The progress of some of the private patients was followed for years after treatment, in some cases for 5 yr or more. In no instance was there any complaint of sequelae or delayed psychopathologic symptoms. The drug ameliorated depression for a period of a few months in a number of patients.

Hundreds of patients have received huge doses of atropine and scopolamine (up to 250 mg), sometimes given three times a week for up to 4 mo, and this form of therapy continues in Eastern Europe today. A chronic behavioral syndrome of toxicity appears unlikely, and single or even multiple exposures to the anticholinergic drugs used in the volunteers, frequently at low doses, are deemed insufficient to stimulate a persistent toxic syndrome. Of course, individual susceptibility to acute effects, which may trigger a long-term effect, cannot be excluded.

The question of occasional long-lasting or late-appearing residual effects is not easily answered. There is very little evidence that these might occur in man, in that so many persons have been exposed to these agents. The new case of glomerulitis several years after exposure in all likelihood was coincidental. Animal studies have been equivocal, but they have suggested the possibility of acute effects on the kidney and liver in some instances.

"Flashbacks" are difficult to explain, but they may result from a heightened sensory awareness after exposure to a drug. They have been of little clinical consequence, even though reported to be associated with a number of mind-altering drugs, including marijuana.

It should be emphasized that careful screening of volunteers for testing with the anticholinergic agents was probably essential, to avoid psychiatric complications after the hallucinatory episode.

If a subject has latent psychopathology, the drug experience may precipitate the latent tendencies and create problems later. In one such instance, a subject receiving Ditran at a university medical school required psychotherapy for 2 mo after the drug episode. Psychiatric interviews and psychologic tests before the drug session indicated that this subject had borderline paranoid and homosexual tendencies; otherwise, he appeared normal. The subject was amnesic during the manifestation of these symptoms; but the complications ensued after other divinity students in his dormitory reminded him of his aberrant behavior under the influence of Ditran. The severe embarrassment resulting from this confrontation may have precipitated the anxiety and loss of self-confidence that required psychotherapy.

In summary, one cannot be certain that long-term adverse effects never follow exposure to anticholinergic agents, but it seems most unlikely.

MORTALITY

The deaths of 222 among some 6,620 soldiers have been analyzed. Table 2-4 shows the standardized mortality ratios (SMRs) comparing the observed numbers of deaths and the numbers expected for the U.S. population categorized by age and calendar year. Most of the SMRs for the various test groups are somewhat less than unity, although many of the groups are relatively small. The values for BZ, scopolamine, atropine, and "anticholinergic only" are 0.87, 2.50, 1.76, and 1.06, respectively. It is interesting that atropine and scopolamine, drugs with a long history of therapeutic use, had SMRs exceeding unity, whereas the four candidate chemical warfare agents (BZ, EA 3834, EA 3443, and EA 3580) had SMRs less than unity. This suggests that exposure to the test anticholinergic chemicals may not be related to a subsequent increase in mortality. For anticholinergic with or without other drugs, an SMR of 0.73 is statistically significant and probably represents a selection artifact, inasmuch as volunteers for these studies were especially screened for good health and thus would be expected to have lower than average mortality.

The mortality data do not reveal a cause for concern that significant numbers of the men exposed to the anticholinergics suffered an untimely death. Because the participants were all especially selected for these studies on the basis of their health records, there were relatively few deaths in all groups. It is impossible to determine how much less than 1.0 the SMR values should have been. Comparison of the values of the various groups would not be expected to reveal treatment effects unless these were sizable, which was not the case. However, a small drug effect might be masked by this type of data analysis. For the same reasons, further subdivision of the deaths by cause does not provide useful data. Mortality is discussed in greater detail in Chapter 4.

SUMMARY

The Panel has examined the assembled data on the possibility of long-term adverse effects associated with the testing of various anticholinergic compounds in human volunteers at Edgewood Arsenal.

Although there are extensive descriptions of the acute CNS effects of the compounds, there is a paucity of information on delayed adverse effects. Animal experiments have not demonstrated significant long-term effects; and chronic administration of other anticholinergic drugs, such as atropine and scopolamine, which have been used therapeutically in man in high doses for years, has not been associated with chronic adverse effects. Indeed, there are anecdotal reports of improvement in behavioral patterns after such treatment. Because the CNS effects of the anticholinergic test compounds appear to be qualitively similar to those of atropine or scopolamine (although they may differ quantitatively in potency and duration of CNS effects), no additional long-term toxicity is anticipated. The apparent lack of an effect on mortality of the test subjects also suggests that such an effect is unlikely. Morbidity data are required to determine whether the subjects have had more health problems than those anticipated in a corresponding control group. This information is not yet available.

In a few persons, some relatively short-term adverse effects were reported after conclusion of the chemical testing (hematuria, hepatitis, and hallucinogenic flashbacks). It is unclear whether these effects resulted from the drug administration or occurred randomly and coincidentally. The extremely low incidence of such reports makes it doubtful that the anticholinergic test substances played a role in inducing these effects. However, the data are not sufficient to answer this question with certainty.

CONCLUSIONS

No firm evidence has been seen that any of the anticholinergic test compounds surveyed produced long-range adverse human health effects in the doses used at Edgewood Arsenal. More intensive study is required to confirm this conclusion. The high frequency of uncontrolled variables makes evaluation of behavioral effects difficult.

On the basis of available data, in the judgment of the panel, it is unlikely that administration of these anticholinergic compounds will have long-term toxicity effects or delayed sequellae. An ongoing morbidity study should provide more definitive information once it is completed.

TABLE 1

EFFECTS OF ATROPINE IN RELATION TO DOSE

Dose, mg	Effects
0.5	Slight cardiac slowing; some dryness of mouth; inhibition of sweating
1.0	Definite dryness of mouth; thirst; increase in heart rate, sometimes preceded by slowing; mild dilatation of pupil
2.0	Rapid heart rate; palpitation; marked dryness of mouth; dilated pupils; some blurring of near vision
5.0	All the above symptoms marked; speech disturbed; difficulty in swallowing; restlessness and fatigue; headache; dry, hot skin; difficulty in micturition; reduced intestinal peristalsis
10.0	Above symptoms more marked; rapid and weak pulse; iris practically obliterated; vision very blurred; skin flushed, hot, dry, and scarlet; ataxia, restlessness, and excitement; hallucinations and delirium; coma

Table 2

Summary of Exposures to Anticholinergics and Records Selected for Review of Acute Effects of Anticholinergic Chemicals[a]

Tox No.	Agent code	No. volunteers	Total exposures[b]	No. records selected
B1	BZ (EA 2277)[c]	292	358	36
B2	EA 3443[c]	101	101	25
B3	EA 3580[c]	130	133	27
B4	Scopolamine	534	636	42
B5	Atropine	444	738	34
B6	EA 3167[c]	2	4	4
B7	Ditran	9	13	12
B8	EA 4929 (benzetimide)	18	18	10
B9	27349	50	50	10
B10	226,086	21	21	8
B11	302,196	52	56	15
B12	301,060	29	29	9
B13	302,282	8	8	8
B14	302,368	5	5	5
B15	302,537	18	18	8
B16	302,668	39	39	11
B18	Benactizine	16	26	9
B21	M-Scopolamine	72	114	21
B22	M-Atro (atropine methyl nitrate)	18	50	10
B23	EA 3834	144	173	33
B25	TAB, BAT	24	24	6
All anticholinergics		1,752[b]	2,614	343

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[a] Chemicals are identified in the master file (Appendix B).

[b] Some volunteers received more than one chemical.

[c] EA 3167, long-lasting; EA 3580, potent; EA 3443, skin penetrant; BZ, chemical-agent candidate.

Table 3

Human Pharmacologic Characteristics of 14 Glycolic Acid Esters
(Based on Edgewood Arsenal Studies, 1960-1971)

Compound	Sources[a] of information	No. subjects	MED[b] (mg/kg)	ID$_{50}$[c] (mg/kg)	Duration,[d] h	Relative Potency[e]	
						Peripheral	Central
226,086	EA report	21	(1.5)	(2.0)	(18-36)	(2-4)	(10.0)
EA 3443	EATR 4066	101	1.2	3.4	48-72	2.3	6.2
EA 3580	231, 265, 269	136	1.4	3.9	16-24	2.6	6.0
EA 3167	248	24	2.8	4.1	(96-240)	(2-4)	(5.6)
BZ	228, App K	354	2.3	5.2	48-72	5.4	4.0
EA 3834	25, 254, 255	171	2.0	5.7	10-15	1.0	3.7
27349	EATM 114-2	50	3.0	6.0	10-15	(2.0)	3.4
301,060	250	29	3.0	(6.0)	(12-20)	(4.0)	(3.4)
302,668	259	39	(4.0)	8.9	10-15	0.9	2.4
302,282	273	20	4.4	12.5	6-10	(2.0)	1.7
Scop	166	637	9.4	20.2	5-10	0.8	1.0
302,196	261	56	(12.0)	28.9	4-6	0.7	0.7
Ditran	166	22	54.0	100.0	4-8	0.2	0.2
MeScopolam.	166	66	5.0	(400)	5-10	3.6	0.5
Atropine	166	602	63.6	152.4	8-12	1.0	0.1

[a] Refers to reference numbers in Appendix I except EA numbers.

[b] Dose required to produce 25% decrement in Number Facility (NF) in 50% of volunteer population (when administered intravenously or intramuscularly).

[c] Dose required to produce 90% decrement in NF scores in 50% of volunteers.

[d] Average time in hours from administration intravenous or intramuscular to near recovery (consistently above 75% of baseline on NF) in subjects receiving ID$_{50}$.

[e] Peripheral potency is in comparison to atropine (based on ED$_{50}$ to produce maximal heart rate of at least 100 beats/min.). Central potency is in comparison with scopolamine (based on ID$_{50}$).

Numbers in parentheses are approximations based on limited information.

Table 4

Observed and Expected Deaths Among Test Subjects by Classification of Chemical Administered and Underlying Cause of Death

	Anticholinergic[a]						BZ only[a]			Scopolamine only[a]			Atropine only[a]		
	Only (N = 570)			Not only (N = 1,179)			(N = 136)			(N = 85)			(N = 62)		
	No. deaths		Obs.	No. deaths		Obs.	No. deaths		Obs.	No. deaths		Obs.	No. deaths		Obs.
Underlying cause of death	Obs.	Exp.	Exp.	Obs.	Exp.	Exp.	Obs.	Exp.	Exp.	Obs.	Exp.	Exp.	Obs.	Exp.	Exp.
All trauma	10	10.0	1.00	11	20.6	0.53	2	3.0	0.67	2	1.3	1.54	1	1.0	1.00
All diseases	5	8.1	0.62	5	14.9	0.34	3	3.9	0.77	0	0.7	-	1	0.7	1.43
Malignant neoplasms	1	1.8	0.56	3	3.3	0.91	0	0.9	-	0	0.2	-	1	0.2	5.00
Cardiovascular disease	3	2.8	1.07	2	4.8	0.42	2	1.6	1.25	0	0.2	-	0	0.2	-
Cirrhosis of the liver	1	0.6	1.67	0	1.2	-	1	0.3	3.33	0	0.1	-	0	0.1	-
Total deaths, all causes[b]	19	18.0	1.06	20	35.5	0.56	6	6.9	0.87	5	2.0	2.50	3	1.7	1.76

	EA 3443 only[a]			EA 3580 only[a]			EA 3834 only[a]			Total		
	(N = 28)			(N = 45)			(N = 64)			(N = 6,620)		
	No. deaths		Obs.	No. deaths		Obs.	No. deaths		Obs.	No. deaths		Obs.
Underlying cause of death	Obs.	Exp.	Exp.	Obs.	Exp.	Exp.	Obs.	Exp.	Exp.	Obs.	Exp.	Exp.
All trauma	0	0.6	-	0	0.8	-	1	0.9	1.11	86	128.2	0.67
All diseases	1	0.5	2.00	0	0.6	-	0	0.4	-	105	146.9	0.71
Malignant neoplasms	0	0.1	-	0	0.1	-	0	0.1	-	30	32.9	0.91
Cardiovascular disease	1	0.2	5.00	0	0.2	-	0	0.1	-	41	58.0	0.71
Cirrhosis of the liver	0	0.0	-	0	0.0	-	0	0.0	-	7	10.5	0.67
Total deaths, all causes[b]	1	1.1	0.91	0	1.4	-	1	1.2	0.83	222	275.1	0.81

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[a] Only: This chemical and none other, except for innocuous and control substances.
Not only: This and one or more other test chemicals.

[b] Includes causes of death listed here and all other causes, including those not yet determined.

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4

MORTALITY

METHOD OF ANALYSIS

The testing of chemical agents in human subjects at Edgewood which began in 1955 continued for more than 20 yr. During that period, the volunteers participated in an average of 3.1 tests each; 29% participated in only one test, and 2% participated in 10 or more tests. Because most of the volunteers participated in more than one test, the date of the most recent test was arbitrarily set as the date on which followup by the Medical Followup Agency of the National Research Council and the ascertainment of mortality began. Followup continued through December 31, 1980. The period of followup ranged from 4 to a maximum of 26 completed years.

The experience of 6,620 men is examined here. One-hundred were excluded from the study because of erroneous identification or because some essential datum, such as date of birth, was unknown. Among those studied, there were 222 deaths.

Deaths during followup were identified by matching the volunteers against a computerized file of veterans maintained by the Veterans' Administration (VA), which includes the date of death of every veteran for whom the VA has received a claim for a burial allowance and other veteran death benefits. Both name and service number were used for matching. If a death certificate showing the cause of death could not be found in the VA claim folder for a deceased veteran, a copy of the certificate was requested from the vital statistics office of the jurisdiction where the death occurred. The underlying cause of death was coded for analysis. The 31 deaths for which death certificates had not been received by the cutoff date for the receipt of data for analysis--March 31, 1982--have been classified as "cause of death unknown." These 31 deaths are distributed in similar proportions between the men who received potentially hazardous substances (15% of 134 deaths) and the men who did not (13% of 88 deaths).

The groups of volunteers cannot be compared for mortality solely on the basis of numbers of observed deaths. One must also take into account the number of years that each group has been followed and the ages of the men being observed. The volunteers in the early test years were about the same age as men used in tests conducted during the later years (Table 4-1). However, the men tested in the early years not only are under observation for a much longer period than men used in the most recent tests, but also have grown older and have had a longer followup. Consider two volunteers of the same age at testing, one whose followup began in 1955 and the other in 1970. The first man will have aged 15 yr by the time the second man is tested and will have been exposed to the risk of dying for an additional 15 yr at the end of followup. Thus, the proportion of volunteers who die during followup will decrease for each succeeding test group, in the absence of any drug effect.

The testing of drugs spanned a period of 21 yr. However, some chemical agents were tested earlier than others (Table 4-2). The testing of LSD and derivatives was concentrated in the early years; more than half the total number of doses had been administered by the end of 1959. At the other extreme were the approved drugs, innocuous substances, and control substances, which were administered late in the testing period. One can therefore expect a much higher proportion of men in the LSD tests to have died than of the men who received approved drugs, innocuous chemicals, or control substances, even if LSD and its derivatives have no adverse effects on survival.

If one calculated the numbers of deaths expected among a test group of men, on the basis of their ages and the numbers of years they were followed, one would have a yardstick for assessing the significance of the number of deaths observed during followup. The ratio of observed deaths to expected deaths, the standardized mortality ratio (SMR), can be used to compare the mortality experiences of two or more groups. Expected deaths by underlying cause were calculated on the assumption that the volunteers experienced U.S. male, age-specific death rates for each calendar year of followup.

RESULTS

Because most of the volunteers participated in two or more tests, an analytic problem arises: If one of the test substances, say Substance A, produced detectable adverse effects, such effects would also tend to appear among men who received Substance B if there were many men tested with both substances A and B. Accordingly, data on men who were tested with any particular substance are shown in two nonoverlapping groups: those who received "only" that substance (whether once or more than once) and those who received "not only" that substance but other active substances as well.

Observed and expected numbers of deaths and the ratios of observed to expected deaths, the SMRs, by underlying cause of death in volunteers who were exposed to chemicals of various types, are shown in Table 4-3. As noted above, a distinction has been made between men who received only one type of chemical and those who were exposed to two or more types (the "not only" groups).

The SMR for the total experience, 0.81, indicates that observed mortality for all causes was only 81% of expectation. That reflects the requirement that a man meet minimal health standards to be accepted into the Army; the screening process results in a death rate that is lower than that of U.S. males in general, and this effect persists for many years. It is evident that men who were tested with active substances had been subjected to additional screening, so these men constituted an unusually fit group and might be expected to have unusually low mortality for a number of years. This phenomenon is identical with the "healthy-worker" effect that is seen in most studies of occupational group. Men who were exposed to two or more types of chemicals experienced lower SMRs than those exposed to only one type of compound, again because of selection, a man had to be in exceptionally good health to participate in multiple tests.

No "all causes" SMR exceeded 1 by an amount greater than can reasonably be attributed to chance. The high SMR, 1.11, representing an 11% excess of observed deaths, occurred in the "no test" group--men who were not exposed to any chemical agent, possibly because of some health defect detected in the examination that preceded testing. The highest SMR, 1.20, occurred in the small group of men exposed only to the cholinesterase reactivators and represents an excess over expectation of less than a single death. The only other SMR to exceed unity occurred in men who were exposed to the anticholinergic chemicals only. Men who were exposed to anticholinergic chemicals plus other chemicals (which sometimes included substances to counteract the effects of the anticholinergic chemicals) had an SMR, 0.56, which is significantly below unity. However, when 212 men exposed to low single doses and 319 to high single doses were examined separately, no dose effect was apparent. Had the anticholinergic chemicals increased the risk of dying, one would have expected that increase to be larger in men with larger single exposures.

No evidence of a deferred, drug-induced mortality increase was seen when the experiences of the first 10 and remaining 16 follow-up years were examined separately. Of the 14 categories of experimental chemicals studied, only two had an SMR exceeding unity during the last 16 follow-up years. One of these (cholinesterase reactivator, only) was clearly due to small numbers, one observed death with 0.8 deaths expected, while the other, 47 observed versus 39.7 expected deaths, was seen in the 1,719 volunteers who were not exposed to any of the chemicals, the "no test" group. We can state with 95-percent certainty that the true SMR for these men during the first 10 years falls between 0.69 and 1.36, while the limits for the last 16 years are 0.83 and 1.54, respectively.

Most of the experimental chemicals were administered to too few volunteers to be examined separately for an influence on mortality. However, a few were considered of sufficient interest to justify being examined in that way (Table 4-4). The specific chemicals are grouped by type. The SMRs for these individual compounds range from a low of 0.80 (nine observed, 11.3 expected deaths) for the men tested with sarin only, to a high of 2.50 (five observed, two expected) for scopolamine only. Atropine, a therapeutic anticholinergic similar to scopolamine, had the next highest SMR, 1.76 (three observed, 1.7 expected). Of the eight deaths of men who received one or the other of these two therapeutically similar compounds, three were attributed to trauma (one accident, one suicide, and one other trauma), and one was due to cancer; the causes of the other four will not be known until the death certificates are obtained.

The SMRs become much more erratic when attention is directed to the underlying causes of death, because of the small expected number. If 0.5 deaths are expected, the SMR will be 0.0, 2.0, or 4.0, if only zero, one, or two deaths are observed.

Trauma was the underlying cause of 86 deaths, compared with 128.2 expected, for an SMR of 0.67. These volunteers had a significantly lower death rate from injury than that experienced by U.S. males in general. This tendency is seen in every category of test chemical, with two exceptions--the 570 men who received anticholinergic agents only (10 observed, 10.0 expected, SMR = 1.00) and the 607 men who received cholinesterase reactivators (11 observed, 11.0 expected, SMR = 100). In each instance, deaths due to homicide contributed to the excess. It would be difficult to attribute an increase in deaths due to trauma to effects of the two types of chemicals, because the excess is not seen among all volunteers exposed to them. No excess is seen in the anticholinergic "not only" group or in the cholinesterase reactivator "only" group.

Three diseases were responsible for more deaths than expected among all volunteers: cancer of the respiratory tract, leukemia and aleukemia, and renal disease. All three renal-disease deaths were in the no-test group; the excess is clearly not a drug effect. The excess of respiratory-cancer deaths derives in part from the control and no-test groups; that suggests that the excess is general, not drug-related. Deaths due to respiratory cancer were responsible for an SMR of 1.11 in a group of World War II and Korean Conflict veterans (1). It is possible that the proportion of cigarette-smokers is higher among veterans than in the U.S. male population in general. The third disease causing more deaths than expected is leukemia and aleukemia (four observed, 2.8 expected, SMR = 1.43). One death occurred in the innocuous-chemical and-control group. Of the other three who died of leukemia or aleukemia, two had received anticholinesterase agents, two cholinesterase reactivators, and one each irritants, cannabis, and an unclassified chemical (included under total experience in Table 4-3). When the total volunteer experience is divided into the 1,984 men who did not receive potentially hazardous chemicals and the 4,636 men who did, the average annual death rates from leukemia are similar--3.4 and 4.0 per 100,000 person-yr of observation, respectively.

What is the chance that an increased risk of death will actually be detected? After choosing a level of significance at which the null hypothesis (no increased risk) will be rejected (P .05, say), the *power* of a comparison is the probability that, in fact, the number of deaths observed will be large enough to constitute a significant excess over the number expected if there were no increased risk.

Power can be large if the relative risk is large or if the sample is sufficiently large. If the sample is small, then only fairly large relative risks will be detected with high probability. In particular, if 10 or more deaths would be expected on the null hypothesis, a relative risk of 2.0 would be detected in more than 80 percent of trials (power greater than 0.80). The same power is achieved if three deaths are expected only if the relative risk is at least 3.0, and if only two deaths are expected at U.S. population rates, the relative risk would have to be at least 4.0 to achieve a power of 0.80. When the number of men exposed to a given chemical are small enough or the condition at increased risk rare enough to produce an expected value of only 0.1 deaths, failure to demonstrate a significant increase would mean only that the relative risk is less than 30. From a statistical point of view, the experience being studied is incapable of demonstrating risks of dying increased less than three- or four-fold.

It can be concluded that, over the time span examined here, there is no evidence that volunteer participation in the testing programs had any long-term adverse effect on mortality.

Table 1

Numbers of Men Tested by Year of Birth and Year of Beginning of Followup

Year of Birth	1955-1959	1960-1964	1965-1969	1970-	Total
	No. Men:				
Before 1920	35	17			52
1920-1924	55	22	2		79
1925-1929	118	78	1		197
1930-1934	417	139	17		573
1935-1939	491	837	77	17	1,422
1940-1944	47	978	805	69	1,899
1945-1949		31	1,191	571	1,793
1950-1954			18	516	534
After 1954			1	70	71
Total	1,163	2,102	2,112	1,243	6,620
	Mean Year of Birth:				
	1934	1939	1945	1950	1942
	Mean Age at Beginning of Followup, yr:				
	23.9	23.5	22.4	22.8	23.1

Table 2

Median Years in Which Categories of Chemicals were Administered

Category	Year
Anticholinesterases	1962
Anticholinergics	1968
Cholinesterase reactivators	1968
Irritants	1967
Cannabis	1965
LSD derivatives	1959
Approved drugs	1971
Innocuous chemicals and controls	1971

Table 3

Observed and Expected Deaths among Test Subjects by Chemical Administered and Underlying Cause of Death

	Anticholinesterase[a]						Anticholinergic[a]						Cholinesterase reactivator[a]					
	Only (N = 507)			Not only (N = 958)			Only (N = 570)			Not only (N = 1,179)			Only (N = 83)			Not only (N = 607)		
Underlying cause of death	Obs.	Exp.	O/E	Obs.	Exp.	O/E	Obs.	Exp.	O/E	Obs.	Exp.	O/E	Obs.	Exp.	O/E	Obs.	Exp.	O/E
All trauma[b]	7	11.7	0.60	13	19.8	0.66	10	10.0	1.00	11	20.6	0.53	1	1.4	0.71	11	11.0	1.00
Accidents	4	7.5	0.53	7	12.5	0.56	6	6.2	0.97	6	12.8	0.47	1	0.9	1.11	5	6.9	0.72
Homicide	1	1.9	0.53	3	3.1	0.97	3	1.6	1.88	2	3.2	0.63	0	0.2	0.0	4	1.8	2.22
Suicide	0	2.1	0.0	0	3.7	0.0	0	1.9	0.0	1	4.0	0.25	0	0.3	0.0	0	2.1	0.0
All diseases	13	17.4	0.75	10	21.9	0.46	5	8.1	0.62	5	14.9	0.34	1	1.1	0.91	4	10.0	0.40
Malignant neoplasms	5	3.9	1.28	5	4.8	1.04	1	1.8	0.56	3	3.3	0.91	0	0.3	0.0	3	2.2	1.36
Respiratory tract	1	1.1	0.91	2	1.2	1.67	0	0.4	0.0	2	0.6	3.33	0	0.1	0.0	1	0.5	2.00
Leukemia, aleukemia	0	0.3	0.0	2	0.4	5.00	0	0.2	0.0	0	0.4	0.0	0	0.0	- -	2	0.2	10.00
Diseases of cardiovascular system	6	7.3	0.82	4	8.4	0.48	3	2.8	1.07	2	4.8	0.42	0	0.4	0.0	1	3.6	0.28
Chronic nephritis, other renal	0	0.2	0.0	0	0.3	0.0	0	0.1	0.0	0	0.2	0.0	0	0.0	- -	0	0.1	0.0
Cirrhosis of the liver	1	1.3	0.77	0	1.7	0.0	1	0.6	1.67	0	1.2	0.0	0	0.1	0.0	0	0.8	0.0
Total deaths, all causes[c]	21	29.2	0.72	28	41.6	0.67	19	18.0	1.06	20	35.5	0.56	3	2.5	1.20	17	21.0	0.81

Table 3 (contd.)

Underlying cause of death	Irritant[a]						Cannabis			LSD derivative[a]			Drugs[a]		
	Only (N = 855)			Not only (N = 1,046)			Total (N = 252)			Only (N = 103)			Only (N = 159)		
	Obs.	Exp.	O/E	Obs.	Exp.	O/E	Obs.	Exp.	O/E	Obs.	Exp.	O/E	Obs.	Exp.	O/E
All trauma[b]	8	16.0	0.50	10	19.6	0.51	1	5.1	0.20	0	2.5	0.0	0	2.4	0.0
Accidents	5	10.0	0.50	7	12.2	0.57	1	3.2	0.31	0	1.6	0.0	0	1.5	0.0
Homicide	1	2.5	0.40	1	3.1	0.32	0	0.8	0.0	0	0.4	0.0	0	0.4	0.0
Suicide	1	3.0	0.33	2	3.8	0.53	0	1.0	0.0	0	0.4	0.0	0	0.5	0.0
All diseases	7	15.0	0.47	9	17.6	0.51	2	4.8	0.42	1	4.6	0.22	0	1.8	0.0
Malignant neoplasms	1	3.3	0.30	4	3.9	1.03	1	1.1	0.91	0	1.0	0.0	0	0.4	0.0
Respiratory tract	0	0.8	0.0	1	0.9	1.11	0	0.2	0.0	0	0.3	0.0	0	1.0	0.0
Leukemia, aleukemia	0	0.3	0.0	1	0.4	2.50	1	0.1	10.00	0	0.1	0.0	0	0.0	- -
Diseases of cardiovascular system	3	5.5	0.55	3	6.3	0.48	1	1.7	0.59	0	2.0	0.0	0	0.6	0.0
Chronic nephritis, other renal	0	0.2	0.0	0	0.2	0.0	0	0.1	0.0	0	0.1	0.0	0	0.0	- -
Cirrhosis of the liver	1	1.1	0.91	0	1.3	0.0	0	0.4	0.0	1	0.3	3.33	0	0.1	0.0
Total deaths, all causes[c]	18	31.0	0.58	23	37.1	0.62	3	9.8	0.31	1	7.1	0.14	1	4.2	0.24

Table 3 (contd.)

Underlying cause of death	Innocuous chemical and control, exclusively (N = 106)			No test (N = 1,719)			Total experience (N = 6,620)		
	Obs.	Exp.	O/E	Obs.	Exp.	O/E	Obs.	Exp.	O/E
All trauma[b]	1	1.7	0.59	31	33.5	0.93	86	128.2	0.67
Accidents	1	1.0	1.00	20	21.1	0.95	54	80.6	0.67
Homicide	0	0.3	0.0	4	5.4	0.74	15	20.5	0.73
Suicide	0	0.3	0.0	2	6.2	0.32	5	24.0	0.21
All diseases	2	1.2	1.67	43	42.4	1.01	105	146.9	0.71
Malignant neoplasms	2	0.3	6.67	6	9.6	0.63	30	32.9	0.91
Respiratory tract	1	0.0	- -[d]	3	2.6	1.15	10	8.5	1.18
Leukemia, aleukemia	1	0.0	- -[d]	0	0.8	0.0	4	2.8	1.43
Diseases of cardiovascular system	0	0.4	0.0	20	17.3	1.16	41	58.0	0.71
Chronic nephritis, other renal	0	0.0	- -	3	0.5	6.00	3	1.7	1.76
Cirrhosis of the liver	0	0.1	0.0	3	2.9	1.03	7	10.5	0.67
Total deaths, all causes[c]	3	2.9	1.03	84	75.8	1.11	222	275.1	0.81

[a] Only: Man received this classification of chemical and none other, except for innocuous and control substances.

Not only: Man received this classification of chemical and one or more other experimental chemicals.

[b] Includes injury undetermined, whether accidentally or purposely inflicted, and injury resulting from operations of war.

[c] Includes the causes of death listed in this table plus all other causes, including cause not yet determined.

[d] Indeterminate, because of zero denominator.

Table 4

Observed and Expected Deaths among Test Subjects by Chemical Administered

Chemical	No. Subjects	No. Deaths Observed	No. Deaths Expected	O/E
Anticholinesterase only	507	21	29.2	0.72
Sarin only	149	9	11.3	0.80
VX only	281	12	14.5	0.83
Remainder of group	77	0	3.3	0.0
Anticholinergic only	570	19	18.0	1.06
BZ only	136	6	6.9	0.87
EA 3443 only	28	1	1.1	0.91
EA 3580 only	45	0	1.4	0.0
Scopolamine only	85	5	2.0	2.50
Atropine only	62	3	1.7	1.76
EA 3834 only	64	1	1.2	0.83
Remainder of group	150	3	3.8	0.79
Irritant only	885	18	31.0	0.58
Mustard only	68	3	3.0	1.00
Remainder of group	817	15	28.0	0.54

REFERENCE

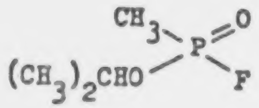
1. Keehn, R. J., 1980: Follow-up Studies of World War II and Korean Conflict Prisoners. III. Mortality to 1 January 1976, Am. J. Epidemiol. 111:194-211.

APPENDIX A

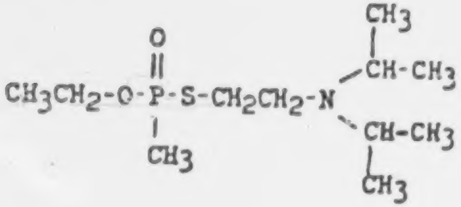
MASTER FILE--ANTICHOLINESTERASE CHEMICALS[a]

Structural Formula

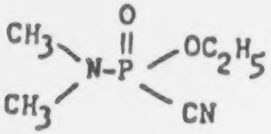
Tox Num A001
  EA Num EA 1208
  Military Desig GB
  Chemical Name Isopropylmethylphosphonofluoridate
  CAS Num 107-44-8
  Common Name Sarin
  Chemical Type Organophosphorus ester



Tox Num A002
  EA Num EA 1701
  Military Desig VX
  Chemical Name Ethyl *S*-2-diisopropylaminoethyl methylphosphonothiolate
  CAS Num 5-782-69-9
  Chemical Type Organophosphorus ester



Tox Num A003
  EA Num EA 1205
  Military Desig GA
  Chemical Name Ethyl *N*,*N*-dimethylphosphoramidocyanate
  CAS Num 77-81-6
  Common Name Tabun
  Common Name Taboon A
  Common Name Trilon 83
  Common Name Gelan I
  Other Num MCE
  Chemical Type Organophosphorus ester



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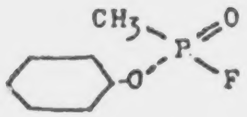
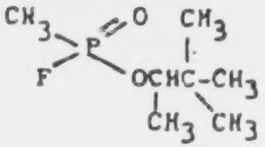
[a] Tox Num = arbitrary NRC designation; EA Num = Edgewood Arsenal designation.

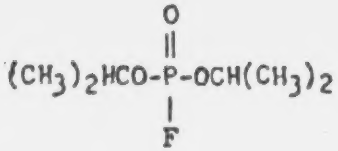
Tox Num A004
  EA Num EA 1212
  Military Desig GF
  Chemical Name Cyclohexyl methylphosphonofluoridate
  CAS Num 329-99-7
  Chemical Type Organophosphorus ester



Tox Num A005
  EA Num EA 1210
  Military Desig GD
  Chemical Name Pinacolyl methylphosphonofluoridate
  CAS Num 96-64-0
  Common Name Soman
  Other Num T.2107
  Chemical Type Organophosphorus ester

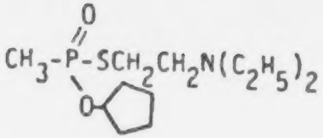


Tox Num A006
  Abbreviated Name - PF3
  EA Num EA 1152
  Military Desig DFP
  Chemical Name Diisopropyl fluorophosphate
  CAS Num 55-91-4
  Other Num T.1703
  Chemical Type Organophosphorus ester

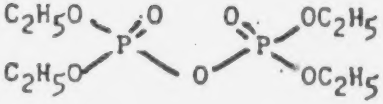


$$(CH_3)_2HCO-\underset{\underset{F}{|}}{\overset{\overset{O}{\|}}{P}}-OCH(CH_3)_2$$

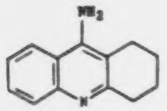
Tox Num A007
  EA Num EA 3148
  Chemical Name Cyclopentyl S-2-diethylaminoethyl methylphosphonothiolate
  Chemical Type Organophosphorus ester



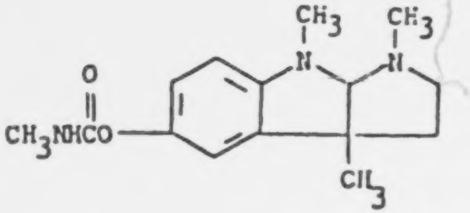
Tox Num A008
  EA Num EA 1285
  Chemical Name Tetraethyl pyrophosphate
  CAS Num 107-49-3
  Common Name TEPP
  Chemical Type Organophosphorus ester



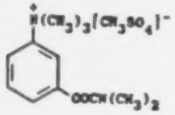
Tox Num A009
Abbreviated Name - THA
Chemical Name 9-Amino-1,2,3,4-tetrahydroacridine
CS Num CS 12602
CAS Num 321-64-2
Common Name Tacrine
Common Name Romotal
Chemical Type Acridine



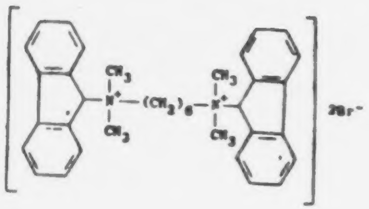
Tox Num A010
Chemical Name Physostigmine
CS Num CS 58525
CAS Num 57-47-6 (free base)
CAS Num 64-47-1 (sulfate)
CAS Num 57-64-7 (salicylate)
Common Name Eserine
Chemical Type Carbamate



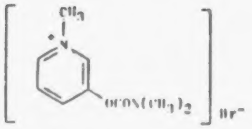
Tox Num A011
Chemical Name *m*-(Hydroxyphenyl)trimethylammonium methylsulfate dimethylcarbamate
CAS Num 51-60-5 (methyl sulfate)
CAS Num 114-80-7 (bromide)
CAS Num 59-99-4
Common Name Prostigmin
Common Name Neostigmine methyl sulfate
Chemical Type Carbamate



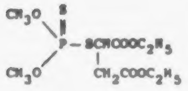
Tox Num A012
Chemical Name Hexamethylenebis[9-fluorenyldimethylammonium bromide]
CAS Num 317-52-2
Common Name Mylaxen
Common Name Hexafluorenium bromide
Chemical Type Curare



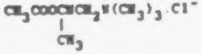
Tox Num   A013
Chemical Name 3-Hydroxy-1-methylpyridinium bromide dimethylcarbamate
CAS Num 155-97-5
Common Name Pyridostigmine bromide
Common Name Mestinon
Chemical Type Carbamate



Tox Num   A014
Chemical Name S-[1,2-bis(ethoxycarbonyl)ethyl]-O,O-dimethyl phosphorodithioa
CAS Num 121-75-5
Common Name Malathion
Chemical Type Organophosphorus ester

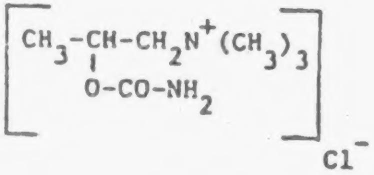


Tox Num   A020
Pharm Class Direct cholinergic
Chemical Name (2-Hydroxypropyl)trimethylammonium chloride acetate
CAS Num 62-51-1
Common Name Mecholyl chloride
Common Name Methacholine
Chemical Type Choline ester



$$CH_3COO\underset{\substack{|\\ CH_3}}{CH}CH_2\overset{+}{N}(CH_3)_3\cdot Cl^-$$

Tox Num   A021
Pharm Class Direct cholinergic
Chemical Name (2-Hydroxypropyl)trimethylammonium chloride carbamate
CAS Num 590-63-6
Common Name Urecholine chloride
Common Name Bethanecol chloride
Chemical Type Quaternary ammonium carbamate



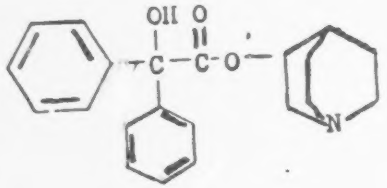
$$\left[CH_3-\underset{\substack{|\\ O-CO-NH_2}}{CH}-CH_2N^+(CH_3)_3\right]Cl^-$$

APPENDIX B

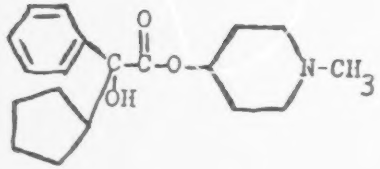
MASTER FILE--ANTICHOLINERGIC CHEMICALS[a]

Structural Formula

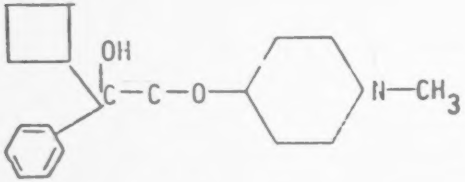
Tox Num: B001
Abbreviated Name: QNB
EA Num: EA 2277
Military Desig: BZ
Chemical Name: 3-Quinuclidinyl benzilate
CS Num: CS 4030
CAS Num: 13004-56-3 (hydrochloride)
CAS Num: 6581-06-2 (free base)
CAS Num: 24572-08-5 (hydrobromide)
Other Num: R02-3308
Chemical Type: Glycolic acid ester



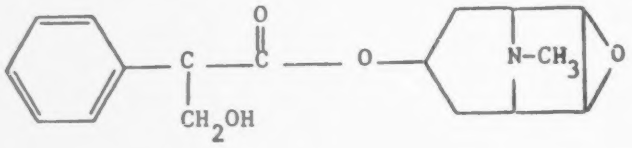
Tox Num: B002
EA Num: EA 3443
Chemical Name: N-Methyl-4-piperidyl cyclopentylphenylglycolate
CAS Num: 37830-21-0
Chemical Type: Glycolic acid ester



Tox Num: B003
EA Num: EA 3580
Chemical Name: N-Methyl-4-piperidyl cyclobutylphenylglycolate
CAS Num: 54390-94-2
Chemical Type: Glycolic acid ester



Tox Num: B004
Cont Num: 300113
CAS Num: 51-34-3 (free base)
CAS Num: 55-16-3 (hydrochloride)
Common Name: scopolamine
Common Name: Hyoscine
Common Name: Sominex
Chemical Type: Glycolic acid ester



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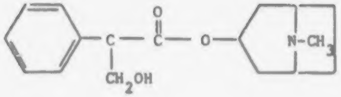
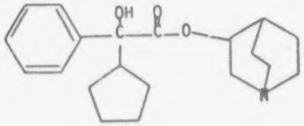
[a] Tox Num arbitrary NRC designation; EA Num Edgewood Arsenal designation; Cont Num contractor's designation.

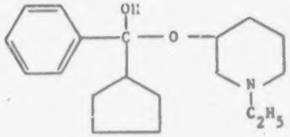
- Tox Num: B005
  - CAS Num: 51-55-8 (free base)
  - CAS Num: 55-48-1 (sulfate)
  - CAS Num: 33952-38-4 (hydrochloride)
  - Common Name: Atropine
  - Chemical Type: Glycolic acid ester



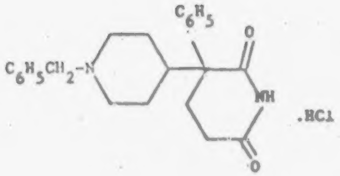
- Tox Num: B006
  - EA Num: EA 3167
  - Chemical Name: 3-Quinuclidinyl phenylcyclopentylglycolate
  - CAS Num: 26758-53-2
  - CAS Num: 29125-55-1 (hydrochloride)
  - Chemical Type: Glycolic acid ester



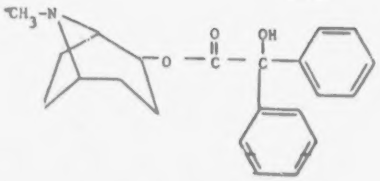
- Tox Num: B007
  - Chemical Name: 1-Ethyl-3-piperidyl α-cyclopentyl-α-phenylglycolate hydrochloride
  - CS Num: CS 4297 (free base)
  - CS Num: CS 62025 (hydrochloride)
  - CS Num: CS 4298 (hydrochloride)
  - CAS Num: 8015-54-1
  - Common Name: Ditran
  - Other Num: JB 329 (free base)
  - Other Num: JB 840 (hydrochloride)
  - Chemical Type: Glycolic acid ester



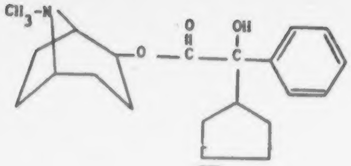
- Tox Num: B008
  - Chemical Name: dl-2-(1-Benzyl-4-piperidyl)-2-Phenylglutarimide hydrochloride
  - CAS Num: 14051-33-3
  - Common Name: Benzetimide hydrochloride
  - Common Name: Dioxatrine
  - Other Num: R4929
  - Other Num: MCN-JR4929
  - Other Num: 4929
  - Chemical Type: Substituted glutarimide



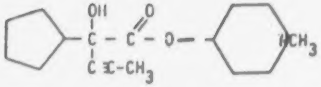
- Tox Num: B009
  - Chemical Name: L-2-α-Tropinyl benzilate hydrochloride
  - CS Num: CS 27349
  - Cont Num: 219758
  - CAS Num: 64520-33-8
  - CAS Num: 64471-12-1 (free base)
  - Chemical Type: Glycolic acid ester



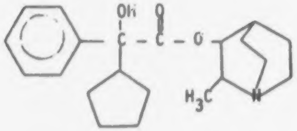
Tox Num: B010
Chemical Name: L-2-α-Tropinyl L-cyclopentylphenylglycolate
Cont Num: 226086
CAS Num: 64471-85-8
Chemical Type: Glycolic acid ester



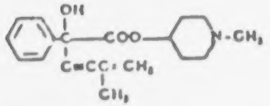
Tox Num: B011
Chemical Name: N-Methyl-4-piperidyl cyclopentyl-(1-propynyl)-glycolate
Cont Num: 302196
CAS Num: 53034-67-6
Chemical Type: Glycolic acid ester



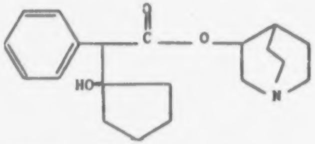
Tox Num: B012
Chemical Name: cis-2-Methyl-3-quinuclidinyl cyclopentylphenylglycolate
Cont Num: 301060
Chemical Type: Glycolic acid ester



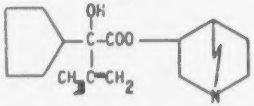
Tox Num: B013
Chemical Name: 1-Methyl-4-piperidyl phenyl-(3-methylbut-1-yn-3-enyl)-glycolate
Chemical Type: Glycolic acid ester
Cont Num: 302282



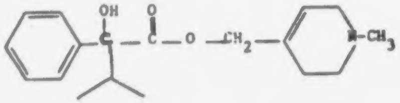
Tox Num: B014
Chemical Name: 3-Quinuclidinyl (1-hydroxycyclopentyl) phenylacetate
Cont Num: 302368
Chemical Type: Glycolic acid ester



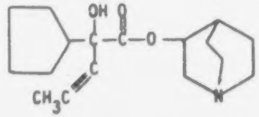
Tox Num: B015
Chemical Name: 3-Quinuclidinyl cyclopentyl-(2-propenyl)-glycolate
Cont Num: 302537
Chemical Type: Glycolic acid ester



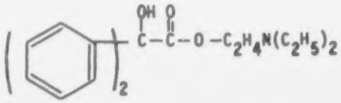
Tox Num: B016
Chemical Name: 4-(1-methyl-1,2,3,6-tetrahydropyridyl)-Methyl-Isopropylphenylglycolate
Cont Num: 302668
Chemical Type: Glycolic acid ester



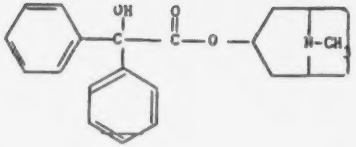
Tox Num: B017
Chemical Name: 3-Quinuclidyl cyclopentyl-(1-propynyl)-glycolate
Cont Num: 302212
Chemical Type: Glycolic acid ester



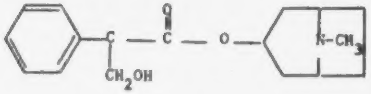
Tox Num: B018
EA Num: EA 2092
Chemical Name: 2-Diethylaminoethyl diphenylglycolate
CAS Num: 57-37-4
Common Name: Benactyzine (hydrochloride)
Chemical Type: Glycolic acid ester



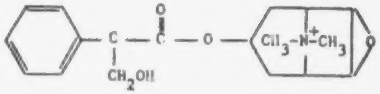
Tox Num: B019
Abbreviated Names: BAT; BETE; BTE
Chemical Name: Tropine benzilate
CAS Num: 3736-36-5
Chemical Type: Gycolic acid ester



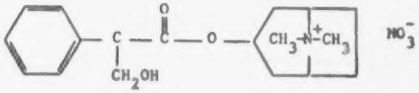
Tox Num: B020
Pharm Class: Anticholinergic
Chemical Name: D-Tropine tropate
CAS Num: 55-47-0 (hydrochloride)
CAS Num: 50700-39-5 (hydrobromide)
Common Name: D-Hyoscyamine
Chemical Type: Glycolic acid ester



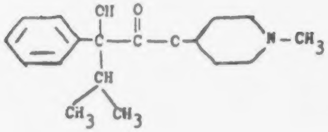
Tox Num: B021
CAS Num: 155-41-9
CAS Num: 6106-46-3 (nitrate)
Common Name: Methyl scopolamine
Common Name: Methscopolamine bromide
Common Name: Pamine (bromide)
Chemical Type: Glycolic acid ester



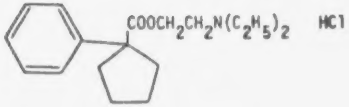
Tox Num: B022
CS Num: 60450
CAS Num: 52-88-0
Common Name: Atropine methyl nitrate
Common Name: Eumydrin
Chemical Type: Glycolic acid ester



Tox Num: B023
EA Num: EA 3834
Chemical Name: N-Methyl-4-piperidyl isopropylphenyl-Glycolate
Chemical Type: Glycolic acid ester

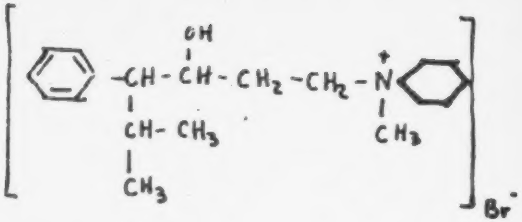


Tox Num: B024
Chemical Name: 2-Diethylaminoethyl 1-phenylcyclopentanecarboxylate hydrochloride
CAS Num: 125-85-9
Common Name: Caramiphen hydrochloride
Common Name: Panparnit
Chemical Type: Basic ester

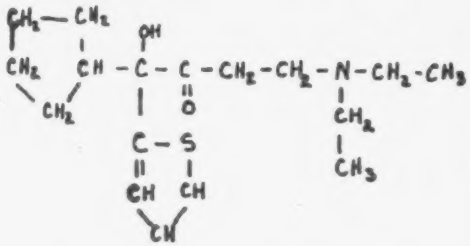


Tox Num: B025
Abbreviated Name: TAB
Common Name: Mixture of TMB4 (D4), atropine (B5), and benactyzine (B18)
Chemical Type: Glycolic acid ester

Tox Num: B026
  Chemical Name: 1-(3-Hydroxy-5-methyl-4-phenylhexyl)-1-methylpiperidinium bromide
  CAS Num: 520-20-7
  Common Name: Darstine
  Common Name: Mepiperphenidol
  Chemical Type: Piperidinium halide


OH
CH - CH - CH₂ - CH₂ - N
CH - CH₃
CH₃
CH₃
Br⁻

Tox Num: B027
  Chemical Name: -Cyclopentyl- -hydroxy-2-thiopheneacetic acid, 2-diethylaminoethyl ester hydrochloride
  CAS Num: 3737-35-7
  Other Num: WIN 2299
  Chemical Type: Glycolic acid ester


CH₂ — CH₂
OH
CH₂ CH - C - C - CH₂ - CH₂ - N - CH₂ - CH₃
CH₂
O
C - S
CH₂
CH CH
CH₃
CH

APPENDIX C

LIST OF DOCUMENTS SUPPLIED TO ANTICHOLINESTERASE PANEL

Background on volunteer testing at Edgewood (use of volunteers in chemical agent research)

Master file and literature references, anticholinesterase chemicals

Research and experimental case file on volunteers (case report summaries, high dose and random samples)

Anticholinesterase chemicals: digest-report

Anticholinesterase chemicals: Karczmar publication

LSD and Himsworth reports (examples of prior long-term toxicity evaluations)

Human test protocols, Edgewood volunteer program

Representative test protocols, random-sample case report summaries

Elements of final report, submitted by Panel members, dated January 21, 1982

Complete list of volunteers (anticholinesterase chemicals) with volunteer numbers (not names), agents, doses, and dates of exposure identified: contains master file and reference file of Edgewood reports

Mortality data evaluation

APPENDIX D

LIST OF DOCUMENTS SUPPLIED TO ANTICHOLINERGIC PANEL

Background on volunteer testing at Edgewood (use of volunteers in chemical agent research)

Master file and literature references, anticholinergic chemicals

Research and experimental case files on volunteers (case report summaries, high dose samples)

Anticholinergic chemicals: digest-report

Anticholinesterase chemicals: Karczmar publication

LSD and Himsworth reports (examples of prior long-term toxicity evaluations)

Human test protocols, Edgewood volunteer program

Representative test protocols, summary of digest-report investigation of two cases of reported flashback

Elements of final report, submitted by Panel members, dated January 15, 1982

Complete list of volunteers (anticholinergic chemicals) with volunteer numbers (not names), agents, doses, and dates of exposure identified: contains master file and reference file of Edgewood reports

Mortality data evaluation

1

APPENDIX E

CASE-FILE DATA ON EDGEWOOD SUBJECTS*

SUMMARY OF VOLUNTEER TESTING WITH GB (A1)

A total of 246 subjects was tested with GB under various conditions. Twenty-five subjects were selected for review, representing approximately 10% of the test group. Selections were based upon the following criteria:

No Treatment (9 subjects)
Route: Intravenous (3.0 - 4.0 μg/kg) 9 subjects
No symptoms
Treatment (PAMCL) (9 subjects)
Dizziness, frontal headache, blurred vision lethargy, nausea, stomach pain, vomiting.
Treatment (7 subjects)
Route: Intravenous (3.0 - 4.0 μg/kg) (P_2S) 2 subjects
Intravenous (3.0 - 4.0 μg/kg) (TMB_4) 3 subjects
Whole-body exposure to vapor (Atropine) 1 subject
Whole-body exposure to vapor (Pyrbenzamine) 1 subject
Rhinorrhea, chest tightness, wheezing.

SUMMARY OF VOLUNTEER TESTING WITH VX (A2)

A total of 740 subjects was tested under various conditions with VX. Seventy-five subjects were selected for review, representing 10% of the test group. Selections were based upon the following criteria:

No Treatment (52 subjects)
Route: Oral (single dose; 3.5 μg/kg) 1 subject
Percutaneous (single dose; 5 - 45 μg/kg) 22 subjects
Oral (multiple dose) 29 subjects
Treatment (23 subjects 2-PAM, Atropine, Regitine
Route: Intravenous (1.2 - 2.0 μg/kg) 7 subjects
Intramuscular 3 subjects
Oral (4.25 - 4.5 μg/kg) 3 subjects
Percutaneous (5.0 - 45 μg/kg) 10 subjects

The vast majority of these subjects reportedly experienced little or no effect from the drug. In 2 cases, the cholinesterase (RBC) level was less that 20% of normal in subjects that received multiple doses, but in each case the dosing was stopped and the cholinesterase levels returned to normal limits within 6-7 days post exposure. No visible effects of the drug were observed in any of these subjects. One subject (A2(BN)) received 30 ug/kg, percutaneously, showed some effects (headache, nausea, ChE 15% of normal, nervous) and was treated with multiple doses of atropine and 2-PAM. Subject recovered and RBCChE was 97% of normal when discharged.

Another subject (A2(BA)) received 1.5 μg/kg intravanously, developed twitching (fasciculation?) of leg muscles, perspiration of palms and soles of feet 15 min after injection when RBC ChE was 87%

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*This represents selection of high-dose subjects. A second set of records of equal number was also procured, on the basis of random selection.

of normal (dropping to 17% at 1h). By 30 min, hands and feet were cold and the eyes felt "heavy." A third subject (A2(BS)), who received 3.2 µg/kg intramuscularly, was dizzy, nauseated, and very drowsy after 2h. During the next 10 hours, the subject lacked concentration and stared vacantly into space. Slight dizziness and blurred vision persisted at 26h when he was treated with 2-PAM and recovered.

Some others reportedly experienced dizziness, light-headedness, nausea, vomiting, "stiff jaw," headache, and ptosis lasting for at least 24h after exposure.

SUMMARY OF VOLUNTEER TESTING WITH GA (A3)

A total of 26 subjects was tested under various conditions with GA. Thirteen (13) subjects were selected for review, representing 50% of the test group. Selections were based upon the following criteria:

Treatment (10 subjects)

Route:
Intravenous (2.0 - 3.0 µg/kg)+(PAMCl, Pretreatment) 3 subjects
Intravenous (1.0 - 2.0 µg/kg)+(PAMCl, Post Treatment) 2 subjects
Intravenous (3.0 µg/kg)+(P_2S, Pretreatment) 1 subject
Intravenous (3.0 µg/kg)+(P_2S, Post Treatment) 2 subjects
Intravenous (3.0 µg/kg)+(PAMCl+TMB4, Pretreatment) 1 subject
Intravenous (3.0 µg/kg)+(PAMCl+P_2S, Pretreatment) 1 subject

All subjects responded to pre- or post-exposure treatment with PAMCl or P_2S when exposed to GF. ChE levels recovered after initial depression within 24 hours or less; they were approaching normal levels by 1 to 2 days post-exposure. Treatment with PAMCl and P_2S or PAMCl and TMB4 did not alter or significantly improve the recovery. None of these subjects reportedly had any serious or prolonged effects from the drug.

SUMMARY OF VOLUNTEER TESTING WITH GD (A5)

A total of 83 subjects was tested under various conditions with GD. Ten (10) subjects were selected for review, representing approximately 12% of the test subjects. Selections were based upon the following criteria:

No Treatment (4 subjects)

Route: Percutaneous (8.5 - 90 µg/kg) 4 subjects

Treatment (6 subjects) Atropine, PAMCl, P_2S, TMB-4

Route: Intravenous (1.0 - 2.0 µg/kg) 6 subjects

Blood cholinesterase measurements were made on the 4 subjects that received GD by the percutaneous route. None of these subjects showed any significant decrease in their ChE levels over a 2-week period. No complaints or severe effects reportedly were observed in any of the subjects tested by this route at the doses used. Two of the subjects who received GD by the intravenous route, although treated with oxime and/or atropine showed significant decrease in the ChE levels, and exhibited the "usual physiological responses" from this agent. Weakness and muscle contractions were also experienced. They also developed persistent vomiting and nausea to a point of dehydration. One subject was referred to Walter Reed Hospital for psychiatric observation and diagnosed for anxiety reaction, acute agitation and hysterical reaction; he was later released for duty.

SUMMARY OF VOLUNTEER TESTING WITH DFP (A6)

A total of 11 subjects was tested with DFP under various test conditions. Five (5) subjects were selected for review, representing approximately 45% of the test group. Selections were based upon the following criteria:

No Treatment (1 subject)
Route: Intramuscular (16.0 µg/kg) 1 subject
Treatment (4 subjects)
Route: Intramuscular (40 - 55 µg/kg) (PAMCl) 3 subjects
Intramuscular (65 µg/kg) (PAMCl + Atropine) 1 subject
(Weak, shaky knees, dizziness, nausea, blurred vision, perspiration).

SUMMARY OF VOLUNTEER TESTING WITH EA 3148 (A7)

A total of 32 subjects was tested under various conditions with EA 3148. Sixteen (16) subjects were selected for review, representing 50% of the test group. Selections were based upon the following criteria:

No Treatment (13 subjects)
Route: Intravenous (0.7 - 1.15 µg/kg) 13 subjects
Treatment (3 subjects)
Route: Intravenous (0.7 µg/kg) (PAMCl) 1 subject
Intravenous (1.15 µg/kg) (Scopolamine) 2 subjects

Subjects with large doses experienced a rapid, severe depression of RBC ChE following agent administration. For example, volunteer #3799 (case #1385), who received 1.15 µg/kg EA 3148, showed 22% of normal RBC ChE values at 15 min after dosing and 0% after 48h; this recovered to 88% of normal at 72d post-exposure.

Of the subjects that received no additional treatment, two subjects who had received the highest dose (1.15 µg/kg) showed toxic signs with 5 to 8 minutes post-exposure. These subjects felt dizzy, weak, tired, sweating, with hands and feet very moist. Within 2 hours post-exposure, these subjects reportedly were resting, eating well and feeling fine. Anorexia, poor sleep, fatigue, unusual dreams, dizziness, euphoria, blurred vision, increased salivation, restlessness are recorded in an Army report summarizing the experience with EA 3148 (CRDL. Tech. Memo 2-31). The report also notes one individual whose schizoid personality seemed to be exaggerated by the drug. Four individuals had decrements on the test of numerical facility.

Subjects treated with PAMCl or Scopolamine did not have any severe drop in ChE, and there were no long lasting effects of the drug observed.

SUMMARY OF VOLUNTEER TESTING WITH THA (A9), Tacrine

A total of 15 subjects was tested under various conditions with THA. Five (5) subjects were selected for review, representing approximately 33% of the test group. Selections were based upon the following criteria:

No Treatment (2 subjects)
Route: Intravenous (24 mg, total) 1 subject
Oral (250 mg, total) 1 subject
Treatment (3 subjects)
Route: Oral (192-200 mg, total) (Atropine) 2 subjects
Oral (250 mg, total) (PAMCl+Atropine) 1 subject

The subjects' blood cholinesterase levels were monitored continuously, along with EEG, EKG, respiratory rate and volume with pneumotact, and blood pressure. There were no significant changes in the parameters measured. Untreated subjects were asymptomatic at the dose levels given, and even though there was some drop in ChE (28% of normal) in plasma, the RBC ChE was unaffected. Subjects treated with atropine alone, or with PAMCl, responded well to relief of severe parasympathomimetic reactions. No residual effects of the drug reportedly were observed in any of the subjects tested.

SUMMARY OF VOLUNTEER TESTING WITH PHYSOSTIGMINE (A10)

A total of 138 subjects was tested with physostigmine and 22 were selected for review, representing approximately 16% of the test group. The criteria for selection was based upon the following: Dose, Route, Frequency and Treatment.

No Treatment (6 subjects)
Route: Intramuscular, single dose (28.0 µg/kg) 2 subjects
Intramuscular, multiple dose (15-30 µg/kg) 4 subjects
Treatment (16 subjects)
Route: Physostigmine in single (45 µg/kg) and multiple doses over 1 to 2 days was administered intramuscularly in subjects that had been previously dosed with a variety of drugs such as:
BZ (7.4 - 14.5 µg/kg) aerosol inhalation, 2 subjects
3834 (2.0 mg) percutaneous, 1 subject
Atropine (125 µg/kg) intramuscular, 3 subjects
Prolixin (15.0 - 23.0 µg/kg) intramuscular, 6 subjects
302668 (10.0 µg/kg) intravenous, 1 subject
302196 (75.6 µg/kg) oral, 1 subject
TAB (90 mg total) intramuscular, 1 subject
Pretreatment with methyl scopolamine (1.0 mg) 1 subject

Only 2 subjects (A10J) and (A10K) who received doses of BZ and were subsequently treated with physostigmine, showed any prolonged central effects (hallucinations, disorientation, confusion) lasting 4 to 6 days post-exposure. Both subjects were asymptomatic and appeared normal when discharged from test. One subject (A10-0) was exposed to Prolixin (23.0 µg/kg) and then treated with multiple doses (1.0 mg x 7 doses) over a 2-day period, intramuscularly: At 27 hours post-exposure, the subject complained of blurred vision, and facial expression was mask-like, tongue "thick" and jaws open. By 28 hours post-exposure, subject was having an oculo-gyric crisis, mouth to one side, and left foot tremulous and turned inward.

Subject was further treated with Cogentin, intravenously (1.0 mg and 1.3 mg), in two doses. Within 30 minutes post-treatment, subject was relaxed and dozing and, by 31 hours post-exposure, the subject appeared normal. At 50 hours post-exposure, the subject was asymptomatic and was discharged at 73 hours post-exposure with no complaints.

SUMMARY OF VOLUNTEER TESTING WITH PROSTIGMINE (A11)

A total of 27 subjects was tested with prostigmine as a part of the esophageal motility studies. Seven (7) subjects were selected for review, representing approximately 26% of the test group. Selections were based upon the following criteria:

No Treatment (1 subject)
Route: Intramuscular (0.5 mg) 1 subject
Treatment (6 subjects)
Route: Intramuscular (1.5 mg) (Atropine/PAMCl) 6 subjects

The esophageal motility studies (EPP) were performed by the subjects before and after administration of the drug. ChE levels were also determined in many of the subjects. One subject developed severe cramps in the abdomen, necessitating termination of the study (A11B). All other subjects reportedly tolerated all procedures very well.

SUMMARY OF VOLUNTEER TESTING WITH HEXAFLUORENIUM (Mylaxin^R) (A12)

A total of 11 subjects was tested with Mylaxin as a part of an esophageal test. 11 Subjects were selected for review, representing 100% of the test group. Selections were based upon the following criteria.

No Treatment (11 subjects)
Route: Intravenous (0.4 mg/kg) 11 subjects

The majority of subjects tested experienced a sensation of "warmness" in the stomach and nausea very shortly (2-10 minutes) after receiving the drug, followed by vomiting within 5-15 minutes. The plasma ChE levels in all subjects were depressed while RBC ChE levels were not significantly affected. Esophageal motility studies disclosed marked spasm of the lower 2/3 during deglutition. This coincided with depression of the plasma ChE. There were no prolonged or long-lasting effects reported in any of these subjects as a result of this drug. All subjects reportedly had recovered within 24 hours post-exposure.

SUMMARY OF VOLUNTEER TESTING WITH MALATHION (A14)

A total of 10 subjects was tested with 1.1% malathion powder dusted over the entire body for up to 10 days. Five (5) subjects were selected for review, representing 50% of the test group. All of these subjects reportedly were asymptomatic and developed no signs of intoxication. One subject (A14A), showed low RBCChE which could not be ascribed to laboratory error; however, there were no other significant drops in this subject's ChE levels. All other subjects showed no significant decreases in cholinesterase.

SUMMARY OF VOLUNTEER TESTING WITH METHACHOLINE (A20)

A total of 9 subjects was tested with mecholyl alone or with EA 1729. Several of these subjects also received other drugs such as neostigmine, urecholine, PAMCl, epinephrine by various routes. Three (3) subjects were selected for review, representing 33% of the test group.

Although these subjects were tested or treated with several of these drugs, as well as mecholyl, their vital signs reportedly returned to normal and they were all in good condition by the end of the testing period. There were no prolonged or delayed effects from this drug reported in these subjects.

SUMMARY OF VOLUNTEER TESTING WITH URECHOLINE (A21)

A total of 15 subjects was tested with urecholine under various conditions, under the (EPP). Five (5) subjects were selected for review, representing 33% of the test group. Selections were based upon the following criteria:

No Treatment (2 subjects)
Route: Subcutaneous (5.0 mg) (single dose) 1 subject
Subcutaneous (5.0 mg) (multiple dose) 1 subject
Treatment of Multiple Compounds (3 subjects)
Route: Subcutaneous (5.0 mg) (PAMCl+Prostigmine) 1 subject
Subcutaneous (5.0 mg) (Prostigmine+Curare+PAMCl) 1 subject
Subcutaneous (5.0 mg) (Tubocurare+Prostigmine+Atropine) 1 subject

EPP were performed before and after administration of the drug. ChE was also monitored in some cases. All subjects tolerated these procedures very well, and there were reportedly no untoward effects of the drugs in any of the subjects tested.

APPENDIX F

EXCERPTS FROM

BZ DATA

Contract DA-18-108-405-CML-826

Hazelton Laboratories
Falls Church, Virginia

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  b. Conditional Avoidance Response Test - Dogs
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APPENDIX G

SUMMARY OF ACUTE- AND CHRONIC-TOXICITY DATA IN VARIOUS MAMMALS WITH THE ANTICHOLINERGIC TEST AGENTS (DITRAN, BENACTYZINE, EA 2545, EA 3167, EA 3443, EA 3392, EA 3580, EA 3834, and 226,086)

by

Leo G. Abood, Ph.D.*

Studies were carried out by Lakeside Laboratories (now incorporated into Merrill Dow Pharmaceuticals, Inc.) in rodents and dogs. The results can be summarized as follows.

TOXICITY

Acute. The intravenous LD_{50} for male rats was 28 mg/kg, and that for male mice was 45 mg/kg.

Chronic. At 8 mg/kg (twice a day) for 8 wk, there was no change in weight of rats; at 300 mg/kg, there was a 12% decrease in weight, compared with controls. In dogs there was no significant change at 3.75 mg/kg.

BLOOD (RATS)

At 300 mg/kg, hemoglobin was reduced by 8%, compared with controls. Sedimentation rates increased by a factor of 3. There was a slight shift in the lymphocyte-to-neutrophil ratio. Leukocyte count increased by about 50% over 8 wk. Terminal red cell count was reduced by 21%.

HISTOPATHOLOGY

At 300 mg/kg, very few abnormalities were observed in rats. An increase in heart weight occurred in some rats; other organ weights remained normal. There were no histopathologic signs at 300 mg/kg; occasional slight changes were due to infection.

In dogs, there were no histopathologic signs attributable to Ditran at 37.5 mg/kg (highest dose). The blood picture was essentially unchanged.

OTHER DRUGS

Acute- and chronic-toxicity tests were performed by various qualified laboratories at Edgewood laboratories on the various test agents used in the Edgewood study in human volunteers. Acute toxicity was usually studied in mice, rats, cats, dogs, and monkeys to determine the LD_{50} and to observe pharmacologic effects, such as autonomic changes, changes in motor activity, ataxia, prostration, and convulsions. All the compounds were similar in the spectrum of their pharmacologic effects and differed mainly in relative potency and

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*Professor, Center for Brain Research, University of Rochester School of Medicine and Dentistry, Rochester, New York 14642.

LD_{50}. For the most part, their LD_{50}s corresponded to central nervous system potency.

Chronic toxicity was usually studied over a period of a month, with single daily injections of at least 3 doses of the test agent, the largest dose being near the LD_{50}. Essentially no gross pathologic effects were noted at any of the doses. Occasionally, at nearly lethal doses, slight changes were observed in the weights of some organs--liver, heart, spleen, and thymus. There were also slight changes in blood cells (e.g., increased leukocyte counts) and the Paneth cells of the intestine (decreased counts).

CONCLUSION

On the basis of the acute- and chronic-toxicity data, the agents were deemed to be safe for human trials, particularly for acute studies of the type performed at Edgewood. It should be noted, however, that no attempt was made to observe any long-term behavioral effects, after either acute or chronic drug administration. Behavioral studies are difficult to perform, requiring a battery of complex operant and psychophysical measuring devices. Even with behavioral testing, it is questionable whether subtle long-range behavioral changes could be detected in animals, particularly in acute studies.

APPENDIX H

EVALUATION OF DATA FROM SHORT-TERM GENETIC TESTING OF ANTICHOLINERGIC CHEMICALS

by

Virginia C. Dunkel, Ph.D.*

A number of short-term tests can be used to determine the genotoxic potential of chemicals. These tests use both prokaryotic and eukaryotic cells and measure such end points as gene mutations, chromosomal aberrations, and interactions with critical macromolecules. It is widely recognized that no test can detect all genotoxic compounds, and multiple end points are required to provide a reliable assessment of genotoxicity. Information from several tests can be combined to reveal two important toxic effects: carcinogenesis and mutagenesis.

There are reports of studies in which short-term tests have been used to determine the genotoxic potential of the anticholinergic compounds 3-quinuclidinyl benzilate (BZ), atropine, and scopolamine. The objective of this discussion is to evaluate the data available on these compounds, to determine whether they are genotoxic. Such information would aid in the overall assessment of the potential of these drugs to produce long-term adverse health effects.

3-QUINUCLIDINYL BENZILATE

In the reported studies of (BZ), the test systems used have had three different end points: point mutations, chromosomal aberrations, and dominant-lethal effects.

In the point-mutation studies (1), _Saccharomyces cerevisiae_ was used as the target cell, and the effects of the chemical were tested in a direct in vitro assay without metabolic activation and in a host-mediated modification. In the direct reverse-mutation assay, there were increases by a factor of 2-6 in the number of His^+ revertants and by a factor of 2-7 in the number of trp^+ revertants over the spontaneous frequencies. In contrast, there were no reported increases in the number of His^+ or trp^+ revertants in the host-mediated assay.

Cytogenetic analyses for chromosomal aberrations were carried out on cells from mice (1) and Chinese hamsters (12) treated with BZ and on human peripheral lymphocytes (1) treated in vitro. It was reported that BZ did not induce translocations and did not increase the number of abnormal metaphases in spermatocytes from male mice.

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With bone marrow cells from treated mice, an increase in the frequency of abnormal metaphases was reported, but there is no information on the type of abnormality observed. Similarly, in the studies with human peripheral lymphocytes, "aberrant cells" were observed at a single concentration of BZ (10^{-4} M), but again the type of aberration was not identified. Lower concentrations had showed no effects, and higher concentrations were reported to be toxic. In the studies with bone marrow cells from BZ-treated Chinese hamsters, Sram (2) reported that there was a dose-related increase in the number of gaps and that the frequency of breaks was increased, but not dose-related. That the occurrence of gaps is not a useful measure of the clastogenic potential of a chemical has been substantiated in other studies (3). In addition, although it was concluded that the frequency of breaks observed was not dose-related, it appears from the data that there were increasing numbers with increasing dose, which plateaued and then began to decrease at the highest doses tested.

The final system used in the evaluatin of BZ was the dominant-lethal assay (1). The only effect observed in these studies was a decrease in the fertility of the males at the highest dose tested. Such an effect is indicative only of the toxic effects of the test compound.

Overall, at most a marginal response might be indicated by the results of the point-mutation assays with Saccharomyces cerevisiae and the possible presence of cytogenic effects--breaks in Chinese hamster bone marrow cells. The significance of these effects, however, is open to question, and further studies are required to confirm or negate the original observations. Since these studies were reported, a substantial amount of information has been obtained on the predictive capacity of short-term tests, and other assays, such as of gene mutation in bacteria and mammalian cells and of unscheduled DNA synthesis, might provide a better base of information for reaching a conclusion about the potential genotoxicity of BZ.

SCOPOLAMINE

The studies reported on scopolamine are also limited and inconclusive, even though the capacity to induce DNA damage and point mutations in bacteria and chromosomal aberrations in mammalian cells has been tested.

No effects were observed in either the DNA-damage assay (4) or the point-mutation assay in Salmonella (5), but the data available on neither of these systems can be considered sufficient for evaluation. In the DNA-damage assay, the compound was treated at only two doses and only in the absence of an exogenous metabolic activating system. The testing in Salmonella typhimurium (the Salmonellamicrosome (test), although conducted both with and without metabolic activation, was at a single concentration, and only two test strains were used. In testing of a compound for its ability to induce mutations in this assay, it is necessary to use a range of concentrations and at least four of the five test strains (6).

In the studies carried out to determine whether scopolamine had the capacity to induce chromosomal aberrations, three different types of mammalian cells were used: HeLa and BSC-K cell lines and human peripheral leukocytes (7). No effects were observed in either the human leukocytes or the BSC-K cells, but chromatid aberrations were observed in HeLa cells at a final 1% concentration of the compound. The aberrations were gaps and breaks, but there was no evidence of exchange figures, such as translocations. As indicated previously, the presence of gaps is not a significant measure of clastogenic potential, and the presence of breaks in a single cell line does not establish the mutagenic potential of a chemical.

The available data on scopolamine are insufficient, and a conclusion cannot be drawn as to whether this chemical is potentially genotoxic.

ATROPINE

As is the case with BZ and scopolamine, reports are available on the testing of atropine for its capacity to induce DNA damage and point mutations in bacteria and chromosomal aberrations in mammalian cells. With one exception, these studies are deficient in one respect or another, and no definitive conclusion can be drawn from them. The problems include lack of incorporation of a metabolic activation system in assays in which the target cell does not have full metabolic capabilities (4,8); lack of a range of doses up to and including one at which toxic effects are observed, that reaches the level of solubility, or that is recognized as sufficient for testing (4,9); lack of specification of the dose used (10); and inclusion of a test method, induction of chromosomal aberrations in grasshopper spermatocytes (11), with which experience in genetic toxicology is limited.

In the single study in which there is adequate information, McCann et al. (12) reported that atropine does not induce point mutations in the Salmonella/microsome assay. The compound was tested at up to 5,000 μg/plate, both with and without metabolic activation, in test strains that can reveal both base-pair and frameshift mutations. This negative result, however, does not in itself provide sufficient information to justify a conclusion about the genotoxic potential of the compound.

CONCLUSIONS

The lack of adequate data, including test results, and limitations in the test systems used preclude a definitive assessment of the genotoxic potential of BZ, scopolamine, and atropine. Only through further studies can a conclusion be reached about the genotoxic potential of these chemicals.

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APPENDIX I

DIGEST REPORT--ANTICHOLINERGIC CHEMICALS

Introduction

Esters of Tropic Acid

- Studies in Animals
- Human Data from General Literature
- Human Data from Edgewood Arsenal
- Summary

Esters of Benzilic Acid

- Introduction
- Data from Experiments with Animals
- 3-Quinuclidinly Benzilate (BZ or EA 2277)
- Diethylaminoethyl Benzilate
- Effects on Man
- Human Data from Edgewood Arsenal
- Summary

Esters of Phenylcyclopentylglycolic Acid

- *N*-Methyl-4-piperidinyl-(phenylcyclopentyl)-glycolate (EA 3443)
- Ditran (CS 4297)
- 3-Quinuclidinyl-(phenylcyclopentyl)-glycolate (EA 3167)
- L-2-alpha-Tropinyl-L-(phenylcyclopentyl)-glycolate
- *cis*-2-Methyl-3-quinuclidinyl-(phenylcyclopentyl)-glycolate (301,060)

Esters of Phenylisopropylglycolic Acid

- *N*-Methyl-4-piperidinyl-(phenylisopropyl)-glycolate (EA 3834)
- 4-(1-Methyl-1-1,2,3,6-tetrahydropyridinyl)-phenylisopropylglycolate (302,668)

Esters of 1-Propynylcyclopentylglycolic Acid

N-Methyl-4-piperidyl-(phenylcyclobutyl)-glycolate (EA 3580)

Miscellaneous Esters

- 302,282
- 302,537
- WIN 2299

Nonester Anticholinergic Compounds

- Benzetimide (CAS 14051-33-3)
- Mepiperphenidol

"TAB" Mixture

Discussion

Note: The chemical nomenclature may differ from that given in the master file. The EA or other number serves for identification of the compound in the master file.

APPENDIX I

DIGEST REPORT
ANTICHOLINERGIC CHEMICALS

by
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INTRODUCTION

The Board on Toxicology and Environmental Health Hazards of the National Research Council provided 58 reports that are related to the administration of anticholinergic compounds to human volunteers by personnel and contractors of the Chemical Corps Medical Laboratories at Army Chemical Center, Md., and its successor organizations at Aberdeen Proving Ground, Edgewood, Maryland. Those reports recorded studies of the responses of humans, mostly men, to 19 individual substances and to a mixture of atropine, benactyzine, and the oxime, trimedoxime (TMB-4). Two of the 19, benzetimide (Dioxotrine) and mepiperphenidol (Darstine), are N-alkylated derivatives of piperidine, but the other 17 are esters. Anticholinergic substances not mentioned in these reports, but said to have been administered also to volunteers, are N-methyltropinylphenylcyclopentylglycolate, 3-quinuclidinyl-1-hydroxyphenylcyclopentylacetate, and N-diethylaminoethylphenylcyclopentylcarboxylate.

The 17 esters represented in the 58 reports mentioned above belong to seven series of compounds: esters of tropic acid, of benzylic acid, of phenylisopropylglycolic acid, of phenylcyclopentylglycolic acid, of 3-quinuclidinol, of N-methyl-4-piperidinol, and of N-diethylaminoethanol.

Esters of tropic acid occur in nature and have a comparatively long history of use as drugs. Atropine (DL-hyoscyamine) and its optical isomers, the D- and L-hyoscyamines, and scopolamine (L-hyoscine) and its optical isomer, D-hyoscine, have been obtained from such plants as deadly nightshade (*Atropa belladonna*), jimsonweed (*Datura stramonium*), henbane (*Hyoscyamus niger*), horsenettle (*Solanum carolinense*), and various species of Scopolia. The L isomers of both esters are more potent than the D isomers.

Both esters have peripheral and central anticholinergic activities; i.e., they interfere with the actions of acetylcholine at peripheral neuroeffector junctions on smooth and cardiac muscles and on cells of glands that produce external secretions and at synapses between some neural elements. Scopolamine is especially potent in the last regard, whereas atropine and L-hyoscyamine are especially effective in blocking the action of acetylcholine at peripheral neuroeffector junctions. Even at neuromuscular junctions on skeletal muscles, which atropine usually is considered not to affect significantly, a sufficiently high concentration (10^{-4} M) of this drug in vitro caused a shortening of the duration of end-plate currents (1).

Quaternization of these alkaloids removes much of their ability to penetrate into the central nervous system and to affect its functions. However, the same variation in chemical structure increases the weak ability of these materials to interfere with the action of acetylcholine at the motor end plates of skeletal muscles.

Toxic psychoses and delirium from ingestion of atropine and scopolamine have been known for many centuries; descriptions of the effects of these drugs antedate by long times the recognition of their mechanism of action. For example, henbane was recommended in the Ebers papyrus of about 1550 B.C. for the relief of abdominal distress.

After the first isolation of atropine from plant material in 1832, the actions of this group of alkaloids were identified rapidly. By 1875, Ringer (2) was able to give the following description (excerpted) of the actions of atropine (belladonna):

> Belladonna employed either internally or externally checks or even suppresses the secretion of the glands. This at least is true of the mammary, sudoriparous and salivary glands, and possibly of other glands.
>
> Its influence on the secretion of the submaxillary glands has been fully worked out. This gland receives branches from the chorda tympani nerve which is endowed with two sets of fibres, one acting immediately on the cells, the other causing the blood-vessels to dilate, being vaso-inhibitory. Belladonna acts through the nerves distributed to the cells, for after the injection of atropia, if the chorda tympani nerve is irritated, the vessels of the submaxillary gland become distended as usual, but the gland does not secrete. The paralyzing effect of atropia is antidoted by physostigma, for after the injection of physostigma, irritation of the chorda tympani causes the gland to secrete.
>
> Dropped into the eye, applied to the skin in its neighborhood, or taken by the stomach, preparations of belladonna very speedily produce extreme dilatation of the pupil. This is one of the most characteristic effects of belladonna.
>
> A full dose of belladonna produces great dryness of the tongue and roof of the mouth, extending down the pharynx and larynx, inducing consequently some difficulty in swallowing, with hoarseness, and even dry cough; and a large dose will sometimes induce dryness of the Schneidarian membrane, and dryness of the conjunctiva, with much injection.
>
> Belladonna often relieves colic of the intestines; and is especially serviceable in the colic of children.
>
> After a considerable dose of belladonna, the face becomes much flushed, the eye bright, dry, and injected, the pupil dilated, the sight dim and hazy, while the power of accommodation in the eye for

> distance is lost. The mind and senses are peculiarly affected. The ideas, at first rapid and connected, become incoherent and extravagant; there is often decided delirium, with pleasing illusions. Sometimes the patient is possessed with constant restlessness, keeps continually moving, and cannot be quieted. A kind of somnambulism is occasionally observed; thus cases are recorded where, under the influence of belladonna, the patient for a long time performs the movements customary to his occupation; thus, it is narrated of a tailor that he sat for hours moving his hands and arms as if sewing and his lips as if talking, but without uttering a word.
>
> The delirium may be furious and dangerous, requiring the patient to be restrained; nay, it is recorded of one poisoned by this drug that so violent did he become that he was ordered to be confined in a mad-house.
>
> The first effect of belladonna on the pulse is to increase its quickness, fullness, and force to the extent even of 50 to 60 beats in the minute. . . . Meuriot is of opinion that belladonna paralyses the peripheral branches of the vagal nerve, and by this means accelerates the heart's action.

Ringer had this to say about the effects of hyoscyamus (scopolamine):

> Thus it produces dryness of the mouth and throat, dilatation of the pupil, presbyopia, lightness and swimming in the head, delirium and hallucinations, a drunken gait, and often a strong desire to fight. Sometimes there is aphonia, and often sleepiness, with oppressive disagreeable dreams. A red rash has been observed after large doses. The pulse at first is much lessened in frequency but soon recovers itself, sometimes becoming even quicker than before the medicine was taken.
>
> Hyoscyamus is generally used to produce sleep when opium disagrees. It has also been employed in neuralgia.

It was only after the role of acetylcholine in the functioning of the autonomic nervous system, in neuroeffector transmission to the various entities innervated by that system and to skeletal muscles (3-11), and in synaptic transmission within some areas of the central and peripheral nervous systems (12-17) had been demonstrated that the proximate mechanism of action of atropine, scopolamine, and other compounds of similar activity could be understood. These compounds seem to attach to the same receptors to which acetylcholine usually attaches itself and thus to interfere with the ability of acetylcholine to produce changes in the properties of cellular membranes.

The actions of atropine and scopolamine to block synaptic transmission within some areas of the central nervous sytem and to induce coma were applied to the treatment of mental disease by Forrer (18) and Goldner (19). In 1950, Forrer published an account of a study of 16 schizophrenic patients treated intramuscularly with large doses of atropine sulfate on 3 days of each week for a long period. The doses of atropine sulfate were increased gradually during a course of treatment from 20-32 mg to 212 mg, to maintain the achievement of unconsciousness, with hyperreflexia and development of the Babinski sign, despite a gradually developing tolerance to the drug.

In 1957, Miller *et al.* (20) reviewed the cases of 206 mental patients treated in this general way, including 148 who had been ill for 2 yr or more before treatment. At the time of that report, the technique was to inject atropine sulfate at 3:00 a.m., so that the patient would recover sufficiently from the effect of the drug to be able to eat lunch and take part in organized activities in the hospital during the afternoon and evening of the same day. Four treatments were given each week, instead of the three that had been given initially. The desired state of coma was produced within 30-60 min of intramuscular injection, and spontaneous recovery, with complete clearing of the sensorium, required 6-9 h after the injection. Of 206 patients treated, 115 (55.8%) were considered to have improved moderately or markedly, 60 (29.1%) to be unimproved, and 31 (15.0%) to have improved only slightly. Six months after completion of a course of injections, 115 patients (55.8%) were still improved moderately or markedly.

In a series of about 500 patients treated with large doses of atropine, with about 10,000 individual inductions of coma, there was one death (21). This fatality was attributed to the replacement, without notification of the physician, of the specially trained nursing and technical personnel on the treatment ward with persons who were not thoroughly familiar with the procedures for caring for comatose patients, and particularly the procedures for controlling febrile reactions. The patient died of uncontrolled hyperthermia.

Goldner (19) reported that he had used intramuscular doses of 5-50 mg of scopolamine in treating beneficially an unspecified number of mental patients, but then had switched to atropine because of its greater availability. He reported on a group of 20 patients treated with atropine-toxicity therapy, 13 (65%) of whom were considered to have benefited moderately or markedly. Goldner also gave a limited comparison of the results of electroconvulsive therapy (ECT) and of atropine-toxicity therapy, stating that several patients who had not benefited from ECT improved on substitution of atropine-induced coma.

Wada *et al.* (22) reported on 51 psychoneurotic and psychotic patients treated with intramuscular injections of 10-220 mg of atropine sulfate every other day during a period of 3-7 wk. The average dose was 30-50 mg. The longest period of observation mentioned in the paper was 6 mo. Of the 51 patients, 28 (54.9%) were considered to have improved detectably. The proportion that improved among the psychotic patients (53.3%) was smaller than that among psychopathic and psychoneurotic ones (66.7%). The greatest alterations in symptoms and signs of the patients' diseases were in psychomotor excitement, hallucination, euphoria, anxiety, compulsion, and delusion. Wada et al. stated that improvement, when it occurred, began with the third to the seventh injection and increased with later injections. If no improvement occurred by the tenth injection, it was not likely to appear. The authors concluded that atropine-toxicity therapy is a relatively safe type of shock therapy, but stated that it was successful in only about 53.3% of their psychotic patients and that its efficacy probably had been overestimated by Schwarz (23). Schwarz had concluded that 25% of 155 cases of psychoneurosis and psychosis had improved markedly with atropine-toxicity therapy and that another 32% had improved moderately.

Miller et al. (20) described the phenomena experienced by patients given large doses of atropine to induce a toxic coma:

> The various stages are passed through quietly and comfortably. Neurologically, there are in order: progressive muscular incoordination, decreased pain sensitivity, and hyperreflexia with the development of the Babinski sign. On the psychological side, we observed clouding of the sensorium, disorientation, loss of time-space relationship, distortion of perception by illusions and hallucinations, confusion, and coma which proceeds to but does not go further than the early fourth stage in one and one-half hours after the drug is administered.
>
> Although the sensorium is markedly clouded, there is no cessation of psychological phenomena. Patients undergoing treatment indicate by their actions, and occasionally by word, that psychic processes continue even during the coma. Illusions seem to be experienced, inasmuch as patients attempt to pick up the bedclothing with their fingers and later report that they saw, and attempted to pick up, flowers, bugs, snakes, et cetera. Conversations may be held with absent persons--indicative of an hallucinatory state. With both atropine-induced illusions and hallucinations, highly significant past events in the patient's life seem to provide the major psychic stimuli. Behavior is correlated with psychological phenomena. Circumoral movements, including sucking and illusory "eating" and "smoking," are commonly observed. When a sufficient degree of motor coordination is retained to accomplish the necessary movement, a progressive, coordinated, and purposeful series of events may often be observed. For example, a pantomime of striking a match, lighting a cigarette, and subsequently of smoking that cigarette is not infrequently observed. Affective lability and corresponding rapid alternation of facial expressions suggestive of pain, sadness, querulousness, and euphoria are commonly observed.

This clinical experience indicating that even large and temporarily incapacitating doses of atropine and scopolamine had no persistent deleterious effects on the health of human beings made it seem that the administration of other, possibly more incapacitating, anticholinergics to human volunteers was not unethical or immoral if two conditions had been fulfilled. These were that a compound to be administered to volunteers had been tested adequately in experimental animals to determine that the probability of its having persistent ill effects on health was small and that the volunteers had been informed as fully as possible of the possible effects of the compound. In the cases of compounds that had been administered to man previously, the effects could be described quite accurately; if the only previous recipients had been experimental animals, the description of possible effects had to be somewhat hypothetical but usually could be reasonably accurate.

Before a discussion of the specific groups of anticholinergic compounds, it may be worth while to have a general view of the procedure of the experiments carried out with human volunteers at Edgewood Arsenal and followed also by contractors. The following description is a composite from two sources: a report from Edgewood Arsenal (24) and a report by a contractor (25).

Test subjects were selected in two stages. Groups of possible volunteers were acquainted with the general nature of the experimental program through a briefing, during which they could ask questions about the general conditions and procedures of the studies. Those who volunteered to take part in the experimental program after this general briefing were given thorough medical examinations, including psychologic or psychiatric assessment. The volunteers considered acceptable for a particular experiment were then given a specific briefing and an opportunity to volunteer to take part in that study.

The studies were carried out in air-conditioned areas from which hazards of accidental injury had been eliminated as thoroughly as possible (e.g., glass panels in doors were replaced with wood, electric outlets and cables shielded, and sharp corners padded). Beds and toilet, messing, and recreational facilities were provided within the research areas. The research was conducted under surveillance of qualified physicians, who administered all injections or oral doses of the test substances. When volunteers were exposed to inhalation of vapors or aerosols, the concentrations of these substances in the air to be breathed by the subjects were established by appropriate personnel. Breathing of the contaminated air by the volunteers was observed and supervised by physicians. After an exposure to an agent, the volunteers were observed continuously for 8-24 h, and then intermittently for up to 14 d. In a few cases, followup studies were made 6 mo or more after the exposure.

ESTERS OF TROPIC ACID

This group contains five of the substances said to have been administered to volunteers, but only three of these are mentioned in the 58 reports related to studies of the effects of anticholinergic compounds on human beings: atropine, scopolamine, and methylscopolammonium. The other two substances in this group that were administered to volunteers are D-hyoscyamine and methylatropinium. Atropine and scopolamine appear, respectively, in 18 and 11 reports, whereas methylscopolammonium salts appear in only three.

Atropine and scopolamine differ only in the basic moiety esterified with tropic acid. Scopolamine and the hyoscines have an oxygen bridge inserted between the two carbon atoms in the dihydropyrrole ring of tropine that are not adjacent to the nitrogen atom, forming scopine, whereas atropine and the hyoscyamines have no such bridge. The facts that atropine is a racemic mixture of D- and L-hyoscyamines, that scopolamine is L-hyoscine, and that the L forms of both hyoscyamine and hyoscine have much greater biologic effectiveness than the D forms mean that atropine sulfate, with only 50% of its active isomer, has only about 60% as much active material per unit of weight as scopolamine hydrobromide, despite the addition of an oxygen atom in the bridge in the latter molecule.

STUDIES IN ANIMALS

Several relatively recent reviews of the actions and potencies of the solanaceous alkaloids are available (26-32). Generally, they give a great deal of information about the medically useful or undesirable actions of the compounds without dealing with their toxicity in any quantitative manner. Table I-1 contains a summary of information on the LD_{50} doses for experimental animals of the five compounds in the group of esters of tropic acid, collected from a variety of published sources (33-41) and from internal reports of Bristol Laboratories, Lederle Laboratories, Maltby Laboratories, the Squibb Institute for Medical Research, Strasenburgh Laboratories, the Upjohn Company, and Sterling-Winthrop Research Institute.

Albanus (42) studied the effects of subcutaneous injections of 13 anticholinergic compounds on central and peripheral cholinergic activities in the dog, recording such factors as the appearance of ataxia and of what he called "obstinate progression" (i.e., the failure of the animal to pull back after encountering an obstacle in its path), the light reflex of the iris, salivation, and heart rate. The compounds administered to dogs included atropine, scopolamine, and a methylatropinium salt. A dose of atropine at 0.1 mg/kg produced no significant alterations in the dogs, but a dose of 0.2 mg/kg produced ataxia in three of four dogs and increased their heart rate by 76%. A dose of 0.3 mg/kg made five of five dogs ataxic and caused three of five to engage in obstinate progression. A dose of 0.5 mg/kg rendered seven of seven dogs both ataxic and out of contact with the environment, as indicated by obstinate progression. This dose also abolished the light reflex of the iris, blocked salivation between 45 and 180 min after the dose, and increased heart rate by 89%.

Scopolamine was considerably more active than atropine in this series of experiments, a dose of 0.03 mg/kg producing ataxia in six of six dogs and increasing heart rate by 24%. A dose of scopolamine at 0.05 mg/kg rendered four of four dogs ataxic and caused them to engage in obstinate progression. This dose also resulted in abolition of the light reflex of the iris, paralysis of salivation between 45 and 135 min after the dose, and a 59% increase in heart rate.

The methylatropinium salt was much less effective than either of the native alkaloids. A dose of 2.5 mg/kg produced ataxia in none of four dogs. A dose of 10 mg/kg induced ataxia in five of eight dogs and obstinate progression in three of eight. It abolished both the light reflex of the iris and salivation between 45 min after the dose and the end of the period of observation, at 315 min after the dose. Doubling that dose induced both ataxia and obstinate progression in four of four dogs.

The only study of the subchronic toxicity of members of the group of esters of tropic acid found is one by Boyd and Boyd (43) with atropine. Groups of rabbits were given daily intramuscular injections of atropine sulfate at 44-118 mg/kg for 100 d. The daily dose required to cause death within 100 d was 78 ± 5 mg/kg, whereas that required to cause death within 10 d was 127 ± 9 mg/kg. The Boyds obtained a relationship between the number (y) of daily injections to produce death and the size of the daily dose (x): $\log y = 2.35 - 0.343 \log x$. The principal sublethal effects noticed in the rabbits were (in the approximate order of their appearance): increased intracolonic temperature, decreased drinking, decreased production of urine, and decreased growth.

Three other papers were related to a special type of subchronic toxicity induced by atropine: alteration of cells, embryos, and fetuses exposed to the chemical. Ishidate *et al*. (44) exposed cultures of fibroblasts from Chinese hamsters to atropine sulfate at 250 ug/ml in saline for 48 h. At the end of the period of incubation, 1% of the cells were polyploid. Of cells examined for chromosomal aberrations after incubation with atropine for 24 h, 1% had gaps and breaks in their chromosomes. These percentages of polyploidy and of chromosomal aberration were only slightly greater than those in cultures to which plain saline had been added. Examination of atropine sulfate for carcinogenicity in animals by the method specified in *Survey of Compounds Which Have Been Tested for Carcinogenic Activity* (U.S. Public Health Service Publication 149) and for mutagenicity in bacteria yielded no evidence that atropine had either activity.

Butros (45) explanted 21- to 22-h chick blastoderms onto agar-containing substrates with atropine at 40-100 µg/ml for 24 h. Blastoderms exposed at 40-80 µg/ml had slight but dose-related injury to the developing neural tube. Those exposed at 90 ug/ml had arrested development with pycnosis and slight cytolysis of the cells of the brain wall and with somites that were smaller than normal and that had slightly opaque cells. Blastoderms exposed at 100 µg/ml had their growth arrested and their brain walls wrinkled and folded, the cells evincing early cytolysis. In some of these blastoderms, the neural tube was completely cytolyzed; in others, the neural tube was open at both its ends. Some 85% of the embryos had normal beating heart tubes; some heart primordia were incomplete and had lateral gaps.

Grunfeld and Balazs (46) reported that pregnant rats given daily subcutaneous injections of atropine sulfate at 0.1 or 0.5 mg/kg-d from day 11 to day 18 of pregnancy had low heart rates, hypothermia, and greater than usual slowing of the heart by a dose of pilocarpine for about a week after the end of administration of atropine. The progeny of these rats had tachycardia for 2 wk after birth and mydriasis for a week after their eyes opened. No long-lasting effects on either the dams or the pups were reported.

Scopolamine, added to the culture medium in a Petri dish at 1 mg/plate after incubation with microsomes prepared from livers of rats, produced no change in the cultural characteristics of *Salmonella typhimurium* strains TA 98 and TA 100 (47). In agreement with this finding of nonmutagenicity of scopolamine for bacteria, a study by Vrba (48) with HeLa cells, human leukocytes, and monkey renal cells revealed that scopolamine hydrobromide at a final concentration of 1% in contact with these cells induced aberrations of the chromatids in the HeLa cells, but not in the monkey renal cells or the human leukocytes. Vrba concluded that changes in chromatids in HeLa cells do not prove that a chemical is mutagenic in man.

Campbell and Ramirez (49) gave three pregnant rats daily intraperitoneal injections of scopolamine at 1.2 mg/kg. Three other groups of two pregnant rats each were given nothing other than the usual food and water or daily intraperitoneal injections of physiologic saline or scopolamine at 2.4 mg/kg. All experimental procedures began on the tenth day of pregnancy and were continued until parturition. Some of the offspring of these dams, when 120 d old, were deprived of water for 18 h and then placed in a conflict situation in which they were exposed to continuous electric shock of variable voltage whenever they tried to drink. The voltage required to inhibit drinking was recorded. Offspring of the three dams that had received daily doses of scopolamine at 1.2 mg/kg were said to have tolerated electric shock better than those of dams under either of the other regimens. No data were presented.

Lavallee (50) collected screening data on scopolamine with dogs and monkeys for comparison with the data from experiments with human volunteers performed at or for Edgewood Arsenal. In dogs, the incapacitating dose of scopolamine had been estimated to be less than 100 mg/kg. In monkeys, with only two animals, only a single dose of scopolamine had been used. The incapacitating dose was clearly above that dose, which had been 32 µg/kg. In man, the incapacitating dose in 50% of the subjects was said to be about 10 µg/kg. The agreement between the values for man and for the experimental animals was not good. Indeed, when a series of seven compounds was examined for comparative effectiveness in man and in dogs and monkeys, the comparative grading in man did not agree with those in the other animals.

In addition to the more or less standard actions of anticholinergic compounds due to their ability to interfere with the actions of acetylcholine on muscarinic receptors (resulting in acceleration of the heart; relaxation of smooth muscles in bronchi and bronchioles, intestinal tract, urinary tract, iris and ciliary body of the eye, bile ducts and gall bladder, and some cutaneous blood vessels; and decrease or abolition of secretion by glands of the gastrointestinal tract and skin), these substances may have pronounced effects on the central nervous system, as has been mentioned earlier. A large amount of research with experimental animals has been directed toward elucidation of the basic mechanisms involved in these actions.

Much of the research has been oriented around one of the basic tenets of pharmacology and toxicology: that a chemical entity affects particular structures in the body because they contain particular aggregates and arrangements of molecules and atoms that establish a special affinity between the structures and the chemical (51). As a corollary, the particular aggregate and arrangement of atoms in a molecule of a chemical entity may condition its affinity for a given site, or receptor. Thus, isomeric forms of a molecule may have different affinities for a given receptor, as has been found to be true for the D and L isomers of hyoscyamine and hyoscine (38). Also, fairly small differences between two molecules may make extensive differences in their abilities to react with particular sites in the body. For example, Graham and Lazarus (34) found that methylatropinium nitrate had approximately the same activity as atropine sulfate in preventing cholinergic stimulation of isolated rabbit intestine, but that it was some 3 times as lethal to mice after intraperitoneal injection. They also found that methylatropinium nitrate had a more pronounced inhibitory effect on the rabbit cardiovascular system than atropine sulfate; this difference between the two compounds certainly contributed to, and may explain, the greater lethality of methylatropinium nitrate. There are indications that quaternization of atropine did not alter extensively its affinity for the receptor on intestinal smooth muscle, but did increase the affinity for receptors on components of the cardiovascular system and perhaps other structures.

Schallek and Smith (52) reported that intravenous injection of atropine at 0.01 mg/kg into dogs anesthetized with thiopental had no detectable effect on their EEGs or their cardiovascular systems. A dose of 0.1 mg/kg increased the predominant frequency in the EEGs of three of five dogs and induced tachycardia in two of five. A dose of 1.0 mg/kg decreased the predominant frequency of the EEGs in four of five dogs and caused hypotension in one of five. Still higher doses increased these actions and also induced bradycardia. Similar effects by atropine and scopolamine in curarized cats and monkeys had been reported previously (53, 54), and stimulation followed by depression by atropine and scopolamine of maze-running by rats had been seen by Macht (55).

In 1963, Zvirblis and Kondritzer (56) found that mitochondria isolated from 0.2 g of brain of young adult white rats adsorbed between 8.4 and 12.6% of the [^{14}C]atropine in 4 ml of atropine solutions with concentrations of 0.063-0.500 µg/ml. When the mitochondria isolated from 0.4 g of brain were immersed in the same concentrations of atropine, the adsorption was between 8.0 and 13.5%. If the lower of the last two values is erroneous (it was obtained with the second lowest concentration of atropine) and the correct value should be about 13.3% between the values for the lowest and the next higher concentrations, the range of adsorption by the larger amount of mitochondria would be 11.4-13.5%. Even with substitution of the hypothetical value in the second series, increasing by 100% the mass of brain substance represented by the isolated mitochondria seems to have increased the adsorption of atropine by no more than a mean of about 13%. Aggregation of mitochondria in the higher concentration of these organelles may have reduced the effective surface for adsorption of atropine. In these experiments, Zvirblis and Kondritzer found also that 3-quinuclidinyl benzilate was adsorbed by mitochondria 2.6-3.3 times as avidly as was atropine.

In 1973, Farrow and O'Brien (57) performed a study somewhat similar to that of Zvirblis and Kondritzer, using atropine and muscarone as the adsorbates and various fractions isolated from a homogenate of rat brain as the adsorbents. They found that both adsorbates--but not decamethonium, dimethyltubocurarine, or nicotine--were bound reversibly on mitochondria in a crude fraction. Muscarone was bound to the extent of only about 0.37 times the binding of atropine. Atropine bound especially to fractions that consisted of presynaptic and postsynaptic membranes, membranes of uncertain origin, microsomes, and synaptosomes. It bound also to homogenates of liver and to mitochondria prepared from liver, but not to homogenates of lung or kidney. Muscarone had a similar pattern of binding to various fractions, except that it did not bind to mitochondria from liver.

Hiley and Burgen (58) used [^{3}H]propylbenzilylcholine mustard as a model muscarinic agonist to examine the concentrations of muscarinic receptor in various regions of dog brain. Synaptosomes and membranes prepared from the head of the caudate nucleus, the proreal gyrus, the uncinate gyrus, the suprasylvian gyrus, the lingual and suprasplenial gyri, and the anterior sylvian gyrus had the largest uptakes of the model agonist, whereas those prepared from the dorsal and ventral halves of the lumbar spinal cord, the subcortical white matter, the pes pedunculi, the corpus callosum, and the cerebellar cortex had the smallest uptakes.

Hiley and Burgen (58) measured also the choline acetylase and acetylcholinesterase activities of selected areas of the brain, in an attempt to determine whether the concentration of muscarinic receptors in a location was related to either of these enzyme activities. The amount of the labeled propylbenzilylcholine mustard bound was not well correlated with either the choline acetylase or the acetylcholinesterase activity; there was a modest correlation between binding of the model agonist by synaptosomes and membranes prepared from nine areas of brain and the choline acetylase activities of the same areas, but it was far from precise.

Burgen _et_ _al_. (59) determined that hyoscine was 10 times as active as atropine in vitro in reducing the uptake of [^{3}H]propylbenzilylcholine mustard by synaptosomes from rat cerebral cortex. A methylatropinium salt was only slightly less active than atropine, but had a very flat dose-action curve: an increase in its concentration by a factor of 10 increased the percent change in uptake of the propylbenzilylcholine mustard only from 64% to 67%. These investigators also reported that the muscarinic receptor in cerebral cortices of the mouse, the guinea pig, the dog, the pig, and the macaque behaved very similarly to that in the rat.

In 1974, the finding of Zvirblis and Kondritzer (56) of a particularly large affinity of the muscarinic receptors in brain for 3-quinuclidinyl benzilate was applied to the assay of the quantity of muscarinic receptor in a sample of tissue (60, 61). Intramuscular injection of atropine 30 min before intravenous injection of [^{3}H]3-quinuclidinyl benzilate decreased the binding of the quinuclidinyl benzilate in various regions of the brain. Although the corpus striatum had the highest uptake of the labeled substance, the cerebral cortex was not far behind. The percent decreases by atropine in the uptake of the ligand were approximately equal for the corpus striatum and the cerebral cortex. The hippocampus, which came next in magnitude of benzilate binding, was also next in magnitude of the change in binding of the benzilate caused by atropine. Indeed, in the six-membered series of corpus striatum, cerebral cortex, hippocampus, hypothalamus, pons-medulla oblongata, and cerebellum, there was complete agreement in rank order for benzilate binding and reduction by atropine of benzilate binding. Administration to rats of mecamylamine, phenobarbital, or L-dihydroxyphenylalanine before intravenous injection of $[^3H]$3-quinuclidinyl benzilate had no effect on benzilate binding in the six areas of the brain.

In these experiments, the highest concentration of labeled benzilate bound to protein was in the microsomal fraction of a homogenate of whole brain, and the next highest was in the mitochondrial fraction, the protein of the microsomal fraction binding nearly 2.5 times as much of the label as that of the mitochondrial fraction. The protein of the nuclear fraction bound only about one-twelfth as much of the labeled benzilate as that of the microsomal fraction and about one-fifth as much as that of the mitochondrial fraction. The muscarinic receptor substance(s) in the rat brain appears to be fairly generally distributed in membranes of subcellular components and to be localized especially in the corpus striatum, the cerebral cortex, and the hippocampus, the last of these three binding nearly 3 times as high a concentration of labeled benzilate as the next most active structure in such binding, the hypothalamus.

Because both muscarinic anticholinergic agonistic drugs (scopolamine, isopropamide, and atropine) and muscarinic agonistic drugs (oxotremorine, acetylcholine, methacholine), but not nicotinic anticholinergic drugs (mecamylamine, hexamethonium, and D-tubocurarine) or nicotinic agonistic drugs (dimethylphenylpiperazinium and nicotine), strongly inhibited the binding of labeled benzilate by homogenates of rat brain, binding of this compound was proposed as an indicator of the presence of muscarinic receptors in particular brain structures. In the nine-membered series of corpus striatum, cerebral cortex, hippocampus, superior-inferior colliculi, pons-midbrain, thalamus, hypothalamus, cervical cord-medulla oblongata, and cerebellar cortex, Yamamura and Snyder (61) found, as had Hiley and Burgen (58) with binding of labeled propylbenzilylcholine mustard, that binding of labeled benzilate was not well correlated with either the choline acetylase or the cholinesterase activity of the particular region, but was slightly better correlated with choline acetylase activity than with that of cholinesterase. Farrow and O'Brien (57) had concluded that binding of labeled atropine to various subcellular components of the rat brain could not be accounted for by binding to acetylcholinesterase.

Farrow and O'Brien (57) had found also that scopolamine was a much more potent inhibitor of the binding of labeled atropine to synaptic membranes than a variety of nicotinic anticholinergic and agonistic drugs; the next most active inhibitor used by these investigators had been carbamylcholine chloride, found by Yamamura and Snyder (56) to be less effective than acetylcholine and methacholine in reducing binding of labeled 3-quinuclidinyl benzilate.

In later work, Snyder's group (62,63) used autoradiography to determine more precisely the locations of binding of [^{3}H]3-quinuclidinyl benzilate within the various regions studied earlier. In the cerebral cortex, the most active areas of benzilate binding were the occipital cortex and the cortex of the cingulate gyrus. The cortex in the piriform area was less active in this regard, and that of the frontal pole still less so. In the hippocampus, three strata of the archipallium were found to have approximately equal abilities to bind 3-quinuclidinyl benzilate, but the white matter of the alveus had much less activity of this sort. The striate body also had high binding activity for this ligand, which was particularly striking in the autoradiographs because of the comparatively low binding activity of the adjoining globus pallidus. Thalamic and hypothalamic nuclei had low binding activities for 3-quinuclidinyl benzilate similar to those of the inferior and superior colliculi. Still lower in activity in binding this ligand were cerebellar cortex, medulla oblongata-pons, and the cerebral peduncles. Nerve tracts--e.g., the optic chiasma, the cervical spinal cord, and the corona radiata--had the lowest binding activities evaluated by Snyder et al.

On the basis of the research outlined in the preceding paragraph, one would expect anticholinergic compounds to produce changes in affect and motor and autonomic regulatory activities. Krieger (64) has found that a dose of atropine that was sufficient to block the circadian increase in the plasma concentration of 17-hydroxycorticosteroids during the night did not prevent stimulation of secretion of 17-hydroxycorticosteroids by the adrenal cortex after administration of ACTH or of stressors, such as insulin and piromen. Thus, the effect of atropine seems to have been exerted not on the adrenal cortex, but rather on the pituitary, the controller of activity by the adrenal cortex through modifiable release of ACTH.

Paton and Rang (65), building on indications in previous papers (66-69) that atropine antagonized the action of acetylcholine in tissues by combining reversibly with specific receptors, found evidence of the existence, in the longitudinal muscle of the small intestine of the guinea pig, of two binding sites for atropine with different capacities for it. The equilibrium constant for binding atropine amounted to slightly more than 5.01 x 10^{-7} M, or about 1,180 pmol/g of muscle. For methylatropinium salts, binding seemed to be to one site with reasonably definite binding properties and to an ill-defined series of other sites with much higher equilibrium constants. Methylatropinium was found to be about 2.34 times as active as atropine in blocking the receptors in this smooth muscle from access by acetylcholine.

Yamamura and Snyder (70) found that these muscarinic receptors resembled those in brain in having an affinity for [^{3}H]quinuclidinyl benzilate that was greater than that for atropine, was the same as that for scopolamine, and was slightly less than that for methylatropinium salts. Scopolamine was about 3 times as active as atropine and about 1.7 times as active as a methylatropinium salt in preventing contraction of the longitudinal muscle of the ileum. The activity of 3-quinuclidinyl benzilate in this regard was the same as that of the methylatropinium salt.

Sayers and Bürki (71) determined the activities of a group of 13 psychoactive compounds, including atropine, in four different test systems, among which was assay of interference with binding of labeled 3-quinuclidinyl benzilate to a homogenate of rat brain. The agreement between interference with binding and interference with stimulation by acetylcholine of isolated guinea pig ileum was good The agreements between interference with benzilate binding and interference with production of tremor in mice by intraperitoneal injection of a standard dose of oxotremorine or dilatation of the pupil of the eye of the mouse were less good.

Witter et al. (72) compared the distributions in the rat brain of tritium-labeled atropine sulfate and methylatropinium nitrate injected intraperitoneally at 10 mg/kg. The rats were killed 30 min after the injections. Livers, kidneys, and brains were collected. Neither atropine nor the quaternized alkaloid was bound to a very great extent in basal ganglia, but both were bound rather extensively in pons-pyramidal tract, preoptic area, and cerebellum. The principal disagreements between the localizations of the two compounds were in the septum, where atropine was bound relatively poorly and the quaternized alkaloid was bound comparatively well (atropine was still bound at nearly twice the concentration at which the methylatropinium radical was bound), and in the hypothalamus and the cortex. In the last two areas, the alkaloid was bound comparatively extensively, whereas the atropinium radical was bound comparatively poorly. In all regions of the brain, atropine was bound to an extent that was 1.9-3.5 times that to which the atropinium compound was bound. In both the liver and the kidney, as well as in plasma, there was a higher concentration of the atropinium radical than of atropine itself. There seems to be no general decrease in the ability of the atropinium ion to move in the body, therefore, but rather a relatively poor ability of that ion to penetrate to or into the cells of the central nervous system. This difference between the alkaloid and its quaternized form may be related simply to the lipoid-rich nature of the central nervous system.

In 1952, Michaelis et al. (73) reported that atropine given before or after DFP did not affect acetylcholine concentration in the cortex of rabbit brain and that DFP increased that concentration by more than 100%. Despite this negative report on the influence of atropine on acetylcholine concentration in the brain, Berry and Stotz (74) stated in 1956 that intraperitoneal injection of atropine at 6 mg/kg decreased the mean concentration of acetylcholine in the rat brain from 365 ug/g to 204 ug/g at 30 min after injection. The depressant effect of atropine on acetylcholine concentration in the brain was confirmed by Giarman and Pepeu in two papers (75, 76). In the first, they reported that intraperitoneal injection of equal doses (50 mg/kg) of atropine and scopolamine into rats produced the same mean percent decreases in acetylcholine concentration in the brain within 10 min after injection. A dose of atropine (400 mg/kg) that produced alternation of central excitation and depression, tremors, and convulsions within 10 min produced a slightly smaller mean depression of acetylcholine concentration than the smaller dose. The difference was not significant, however.

In the second paper (76), the investigators compared the effects on brain acetylcholine concentration in the rat caused by a series of doses of atropine sulfate with those caused by two doses of hyoscine hydrobromide and one dose of methylatropinium nitrate at 30 min after intraperitoneal injections of the drugs. The three lowest doses of atropine sulfate (1, 5, and 25 mg/kg) produced dose-related mean decreases in acetylcholine concentration, but the highest dose (50 mg/kg) produced a smaller mean decrease than the two next lower doses. A dose of methylatropinium nitrate equimolar with a dose of atropine sulfate of 2.5 mg/kg resulted in a slightly smaller decrease in the mean acetylcholine concentration than the atropine dose of 1.0 mg/kg. A dose of hyoscine hydrobromide equimolar with atropine sulfate at 2.5 mg/kg resulted in a lowering of the mean acetylcholine concentration nearly midway between those resulting from doses of 5 and 25 mg/kg of atropine sulfate.

In a study of the time course of the decrease in the acetylcholine concentration in the brain in the rat, Giarman and Pepeu (76) found that the maximal change occurred at about 60 min after intraperitoneal injection of hyoscine hydrobromide and that injections of this compound at 1 mg/kg three times per day for 6 d resulted in progressive decreases in acetylcholine concentration during the first 3 d. Later, the concentration increased slightly, so that at 145 h after the first injection of hyoscine hydrobromide (1 h after the nineteenth injection) the mean value was 60% greater than the lowest one, observed at 73 h following the first injection (1 h after the tenth injection). The greatest change in acetylcholine concentration was in the cerebrum (a decrease of 35%), accompanying changes in the rostral mesencephalon and in the caudal mesencephalon, with the metencephalon being below the level of significance.

Aquilonius et al. (77) studied the effects of atropine and of methylatropinium nitrate on the release of acetylcholine by the cortex of the rat brain (by vividiffusion) and on motor activity (jiggle cage). Atropine sulfate and methylatropinium nitrate at 0.25-10 mg/kg were injected into one jugular vein. Although doses of methylatropinium nitrate of 0.25-0.75 mg/kg produced slightly greater release of acetylcholine into the collecting cups than the same doses of atropine sulfate, the former drug in doses up to 10 mg/kg produced a maximal increase in motor activity of 2.33 times basal activity, whereas atropine sulfate produced an increase in motor activity of 6.75 times basal activity.

By using atropine and methylatropinium labeled with tritium, Aquilonius et al. (77) found that injection of equimolar doses of the two compounds into a jugular vein resulted after 30-60 min in nearly equal concentrations of the labels from the two compounds in not only the brain and cerebrospinal fluid but also the plasma. Despite the fairly marked differences between the fractions of the injected doses of the two compounds that entered the cerebrospinal fluid and brain, the fractions of the doses that entered the fluid in the collecting cups were not very different after 30 min; after 60 min, they were somewhat more different, but perhaps not significantly so. To explain the appearance of the methylatropinium compound in the vividiffusion cup and in the cerebrospinal fluid, the investigators adopted the suggestion of Szerb (78) that such charged compounds as the quaternized alkaloid may pass from the blood into the subarachnoid fluid and both seep into a vividiffusion cup and cause superficial layers of cortical cells to release acetylcholine.

Following the finding that septal lesions result in increased concentrations of acetylcholine in the hippocampus, Rommelspacher and Kuhar (79) studied the effects of muscarinic cholinergic agonists and antagonists on the acetylcholine concentration in the rat hippocampus after lesions of the septum had been produced electrolytically. The drugs were injected intraperitoneally just before administration of ether for creation of the septal lesions. The rats were killed 20 min after the lesions had been made. Doses of 5 and 10 mg/kg of atropine and scopolamine resulted in decreases (atropine) and increases (scopolamine) in the mean concentration of acetylcholine in the hippocampus. Smaller doses of atropine (0.1 and 1.0 mg/kg) may have induced increases in the acetylcholine concentration; only two rats received each of these doses.

Berger et al. (80) induced hippocampal after-discharges by electric stimulation of the fornix and found that doses of atropine at 1-2 mg/kg, presumably injected intravenously, doubled the duration of the after-discharges induced by a standard stimulus. This result may have been due to increased release of acetylcholine.

Frances and Jacob (81) found that eserine and arecoline, on the one hand, and atropine and scopolamine, on the other, had oppositely directed effects on the acetylcholine concentration in the mouse brain. Eserine and arecoline induced increased concentrations, whereas atropine and scopolamine induced decreases. These data indicate that a subcutaneously injected dose of scopolamine about one-fifteenth that of atropine administered similarly produced the same decrease in acetylcholine concentration in the brain as the larger dose of atropine. In the case of scopolamine, the percent decrease in acetylcholine concentration was a linear function of the log dose of the alkaloid in the range of doses used--0.3-3 mg/kg. Atropine at 1 to about 8 mg/kg yielded a linear relationship between percent decrease in acetylcholine concentration and log dose. At 10-30 mg/kg, there was no further effect on acetylcholine concentration by atropine, although the maximal change induced by this compound was only about two-thirds that induced by the largest dose of scopolamine used. There was a linear relationship between the percent decrease in acetylcholine concentration and activity (jiggle cage) in rats given scopolamine at 3 mg/kg or atropine at 10-30 mg/kg.

Herink et al. (82) used septal lesions to modify the motor activity of rats, as had Rommelspacher and Kuhar (79). An intraperitoneal injection of atropine at 1 mg/kg in normal rats increased aggressiveness within 5 min, whereas in rats with septal lesions it decreased aggressiveness. Atropine decreased defecation, an index of emotion, in both types of rats.

Usui and Iwahara (83) used freely moving male rats with recording electrodes in the dorsal hippocampus and in contact with the dura over the frontal and occipital cortices and found that atropine at 5-30 mg/kg injected intraperitoneally delayed the first appearance and shortened the first episode of paradoxic sleep. Doses greater than 10 mg/kg usually prevented paradoxic sleep entirely. The regularity of the theta rhythm from the hippocampus was markedly disturbed in paradoxic sleep without rapid eye movements; when paradoxic sleep was accompanied by rapid eye movements, theta activity was increased in both frequency and amplitude. Theta activity associated with body movement was not altered by atropine. The hippocampus seems to have two systems for generating theta activity, one that contains muscarinic cholinergic receptors and is susceptible to modification by atropine, and one that is not susceptible to alteration by atropine.

Takeyasu et al. (84) gave male rats two intraperitoneal injections of atropine at 3 mg/kg each day for 4 wk, after which the rats were not given atropine for 5-8 d. At the end of the withdrawal period, the effect of subcutaneous atropine (1-20 mg/kg) on motor activity was evaluated. Previously unatropinized rats were subjected to the same doses of atropine while their activity was monitored. Both the naive and the atropine-adapted rats yielded dose-related curves of increased activity as the dose of atropine was increased; the curve for naive rats was considerably higher than that for the atropine-adapted ones. Takeyasu et al. found evidence that the masses of muscarinic cholinergic receptors in the cerebral cortex, the hippocampus, and the corpus striatum were all increased by the repeated daily doses of atropine. They suggested that the smaller response to atropine in the atropine-adapted rats than in the naive ones might be due to this factor. (Both curves in Figure 1 of the paper by Takeyasu et al. tend to become parallel to the dosage axis at different levels of activity, rather than converging as required by their hypothesis.)

In dogs, intravenous injections of atropine at 0.5-1.5 mg/kg produced a syndrome of decreased alertness and responsiveness to commands, stumbling and slipping, swaying, incoordination and ataxia, splaying of the hind legs, blundering into obstacles and attempting to progress forward despite the presence of an obstacle, whining or barking, agitated pawing, apparent pursuit of unreal moving objects, collapse, and sleep from which the dogs could be aroused temporarily by handling or noise (85). Recovery from a 1-mg/kg dose of atropine required about 5 h. Intravenous injections of tetrahydro-5-aminoacridine [THA] at 1 mg/kg and yohimbine at 0.5 mg/kg were found to produce some recovery from the effects of atropine, THA being more effective than yohimbine [86]. These authors did not find that small intravenous doses of physostigmine or neostigmine (0.02 mg/kg) were significantly antagonistic to the central effects of anticholinergic drugs.

Because the usual dosage of atropine is small, its metabolism could not be studied effectively until fairly recently, when methods for labeling the drug with radioactive isotopes and for measuring the concentrations of the isotopes in biologic samples became available. Evertsbusch and Geiling (87) seem to have been the first investigators to use ^{14}C-labeled atropine. Their atropine was prepared by extraction from Atropa belladonna grown in an atmosphere of $^{14}CO_2$, so that the position of the label was unknown. The most sensitive method for determining atropine had been the mouse eye assay, with which Pulewka (88) and Tonnesen (89) were able to determine that only a fraction of a dose of atropine administered to man (by mouth or by subcutaneous injection) appeared unchanged in the urine. Evertsbusch and Geiling (90) found that a portion of the label from randomly labeled [^{14}C]atropine appeared in the expired air as $^{14}CO_2$.

In 1953, Gosselin et al. began an intensive study of the metabolic fate of atropine made by esterifying tropinol with tropic acid that contained an atom of ^{14}C in the position between the benzene ring and the carboxyl group. Mice were the first animals studied (over 100 were used), followed by rats, a few cats, and finally eight human beings. The original group of investigators separated into three parts before the entire series of studies was completed, and one of those parts studied the fates of two differently labeled atropines in man.

The first part of the research to be reported was the study of the metabolic fates of labeled tropic acid and atropine in mice and rats (91,92). Mice and rats given intraperitoneal injections of alpha-[^{14}C]tropic acid at 1 mg/kg excreted in their urine only a single labeled compound that had essentially the same chromatographic characteristics as authentic tropic acid. The excretion of ^{14}C administered in this form was complete within about 2.5 h in the mice and within slightly more than 3 h in the rats.

When the labeled atropine was administered to both mice and rats by both intravenous and intraperitoneal injections, the cumulative excretion of ^{14}C in the urine by the mouse was always considerably greater than that by the rat. The label from alpha-[^{14}C]atropine injected intravenously into mice appeared in their urine slightly more promptly and to a somewhat greater extent than that from labeled atropine that had been injected intraperitoneally or subcutaneously or that had been administered by gavage. The curve for the clearance of ^{14}C from the body of the mouse with time elapsed after subcutaneous injection of the labeled alkaloid required three simultaneous exponential equations to represent the observations after a period of latency of about 45 min, during which excretion of ^{14}C followed none of the three exponential relationships. In addition to atropine itself, at least three other substances containing ^{14}C appeared in the urine of the mouse.

At 30 min after intravenous injection of labeled atropine into mice, the structures found to have the highest concentrations of ^{14}C were the gall bladder and the bile, the urinary bladder, and the duodenum and jejunum. By 4 h after injection, the structures with the highest concentrations of ^{14}C were the gall bladder and the bile, the urinary bladder, and the ileum. At 24 h after injection, the structures with the highest concentrations of ^{14}C were the colon and cecum, the gall bladder and the bile, and the stomach. (In all cases, the hollow viscus and its contents are designated by the name of the viscus alone.) The comparatively high concentration of the label in the stomach 24 h after injection may indicate that the mice had ingested some of their feces, the contents of the colon and cecum having high concentrations of ^{14}C at that time. No $^{14}CO_2$ was found in the expired air.

It is clear that the principal routes of removal from the body of the atropine label used by Gosselin et al. (91, 92) were the urine and the feces, the intestinal route of excretion being comparatively minor. Whatever the mode of administration of the labeled atropine to mice, 80-90% of the ^{14}C was excreted in the urine. The consistent presence of the gall bladder and its contents among the structures with the highest concentrations of the label indicates that the label enters the gastrointestinal tract after secretion by the liver into the bile, which then flows into the small intestine through the bile duct and the common duct. The progressive shift of the segment of the intestinal tract with the highest concentration of ^{14}C from the duodenum and jejunum to the ileum and then to the large bowel reflects the movement of a bolus of an especially high concentration of the isotope down the tract during a period of about 24 h.

The failure to find $^{14}CO_2$ in the expired air of the mice indicates that the tropic acid moiety in the atropine molecule is not metabolized--in accord with the finding that labeled tropic acid itself was excreted without loss in the urine. The finding of atropine but no tropic acid in the urine of the mice indicates that hydrolysis of the ester did not occur rapidly. The latter finding was somewhat unexpected because an esterase capable of hydrolyzing atropine is present in the livers of many vertebrate species, although it or a similar enzyme appears in the sera of only a few species (93-95). This enzyme is also able to hydrolyze L-hyoscyamine, tropinyl benzoate, and caramiphen (95).

The rat was found (96) to excrete in its urine substances containing ^{14}C derived from the labeled atropine with approximately the same paper chromatographic retardation factors (Rfs) as those found previously in the urine of the mouse. The rat differed from the mouse in excreting about 40% of the label in its feces, compared with about 10% for the mouse. The guinea pig, unlike the other mammals studied, excreted in its urine a large part of the ^{14}C in a form that was probably tropic acid or some similar molecule (Rf slightly below that for tropic acid added to urine from the guinea pig). The majority of the ^{14}C in the urine of the cat was excreted in the form of atropine, only two other small peaks of radioactivity being evident on paper chromatograms. The rat and the cat, unlike the mouse and the guinea pig, excreted almost as much of the label in feces as in urine. In the rat and the cat, the livers were more active both in initial uptake and in maintaining a high uptake until 4 h after intravenous injection of labeled atropine than those of the mouse and the guinea pig. Increased transfer of the isotope from the blood into the bile by the liver in the former two species probably explains its greater fecal excretion by these animals than by the other two species.

Despite the quantitative differences in excretion of labeled atropine by different routes in the mouse and the rat, Gabourel and Gosselin (97) proposed that atropine follows the same metabolic pathways in the rat as in the mouse. They studied mice given intravenous injections of alpha-[^{14}C]atropine and found that approximately 25% of the dose was excreted in the urine as atropine, more than 50% as glucuronides, and the remainder as intermediate oxidation products and, probably, tropic acid esters with altered tropyl moieties. Although they were unable to prove that the aromatic ring of the tropic acid moiety was hydroxylated, they proposed that hydroxylation of this ring, initially at the 4-position and then at the 3-position, resulted eventually in the production of monoglucuronides and diglucuronides. However, Phillipson et al. (98) found that incubation of atropine or hyoscine with a preparation of microsomes from guinea pig liver led first to the dealkylation of the nitrogen atom of the tropyl or the scopyl moiety and sequentially to production of the N-oxides and N-hydroxynoratropine or N-hydroxynorhyoscine. It seems possible that conjugation with glucuronic acid from uridine-5-diphospho-alpha-D-glucuronic acid under the catalytic influence of uridine diphosphate glucuronyl transferase occurred in the course of the oxidative series of reactions mentioned earlier and resulted in the formation of an N-glucuronide of noratropine. Such a reaction is known to occur with secondary aromatic amines, such as noratropine.

Werner and Schmidt (99) studied the types of chemical reactions undergone by [^{14}C]atropine introduced into mice, rats, guinea pigs, rabbits, cats, and monkeys. Their general conclusion was that, although various species differed quantitatively in the relative amounts of particular end products of atropine metabolism, the chemical changes undergone by the molecule in the various species were the same: hydrolysis of the ester linkage, hydroxylation of the benzene nucleus, glucuronidation, and oxidation to CO_2.

Kalser et al. (100) found that bile of rats into which [^{14}C]atropine had been injected intravenously might contain up to five peaks of radioactivity of comparatively low Rf: 0.02, 0.08-0.09, 0.16, 0.22-0.23, and 0.68. The substances represented by Rfs of 0.08-0.09 and 0.16 were present in the bile in the highest concentrations. No tropic acid or unaltered atropine was found in bile. Metabolites of atropine appeared in the bile within 10 min after intravenous injection of that alkaloid. The plasma of these rats contained only unchanged atropine in concentrations great enough to be detectable.

Isolated, perfused livers of rats exposed to [^{14}C]atropine injected into the perfusion fluid excreted about 60% of the ^{14}C into the bile during 4 h (101). All the metabolites were more polar than atropine itself, but they were not identified.

Winbladh (102) presented data on the distribution of atropine labeled randomly with ^{3}H in newborn pups,

pups at 3 and 6 wk of age, pups at 3 mo of age, and adult dogs (10 mo old or older). After subcutaneous injection of labeled atropine at 0.5 mg/kg, the isotope was removed from the blood plasma much more slowly in the newborn pups than in the adult animals. The pups of other ages had intermediate half-times for removal from the plasma, the greatest step of difference being between newborn pups and those 3 wk old. The half-times for removal of the label from the plasma after intravenous injection of labeled atropine were not strikingly different from those measured after subcutaneous injections.

In newborn pups, the organs that initially took up the highest concentrations of the label were the kidneys and the liver, the kidneys having a higher concentration. The liver maintained a higher proportion of its early concentration of ^3H until 8 h after the injection than the kidneys, however. Pups 6 wk old behaved qualitatively like the newborn ones, but in the adult dogs the initial uptake of the label by the liver was considerably greater (by up to 80%) than that by the kidneys. Of the ^3H in the urine, 50-90% was in the form of unchanged atropine. In brain, Winbladh (102) found a progressively decreasing concentration of atropine (the only form in which the label was found in brain) as serial samples were taken from the cortex to the vicinity of the lateral ventricles.

Harrison et al. (103) used atropine labeled with ^3H in the para position of the benzene ring in the tropic acid moiety of atropine to follow the disposition of that alkaloid in the rat. They found that the mean half-times for removal of the label from various tissues after intraperitoneal injection were as follows: plasma, 44 min; heart and kidney, 54 min; liver, 74 min; brain, 76 min; and fat, 101 min. The half-times were determined from the slopes of the decay curves.

Two fairly recent papers on the metabolic fate of atropine in man have been published (104,105); an excellent summary of the work was presented by Kalser (106). The two subjects in the first of these papers excreted within 24 h 85% of an intramuscularly injected dose of 2 mg of alpha-[^{14}C]atropine. A urine sample collected from one of the subjects between 1.5 and 4 h after injection contained substances that produced four small, interconnected peaks of radioactivity on a paper chromatogram that were apparently removed by incubation of the urine with bacterial beta-glucuronidase. Only three clear peaks of radioactivity appeared on the paper chromatograms of this urine after it had been exposed to glucuronidase. Two of these had Rfs that agreed with those for atropine (the major peak) and for tropic acid (a small peak). The identity of the third peak, with an Rf below that for atropine, was not determined. After alkaline hydrolysis of this sample of urine, the only radioactive substance detected in the chromatogram had an Rf similar to that of tropic acid; thus, the tropic acid portion of the ester seems not to have been altered metabolically to any important extent.

The second paper (105) used two samples of [^{14}C]atropine; one was labeled on the methyl group attached to the nitrogen atom of the tropine moiety, and the other was labeled at the two carbon atoms in the ring structure of the tropine moiety adjacent to the carbon atom involved in the ester linkage. Two male and two female subjects received intramuscular injections of 2 mg of one of these labeled atropines. One subject received individual injections of each labeled atropine on two different days. When the methyl-labeled atropine was administered, $^{14}CO_2$ appeared in the expired air. Administration of the other labeled atropine did not result in the excretion of $^{14}CO_2$, nor had this substance been found in the air expired by the only one of the subjects in the first of these papers (104) whose expired air was examined. One concludes, therefore, that the only part of the atropine molecule that is subject to complete oxidation is the methyl group on the nitrogen atom of the tropine moiety. As for excretion of the label in the urine, the two samples of atropine used by Kalser and McLain yielded similar results. There was rapid excretion of ^{14}C during the first 4 h after injection that was nearly linear with time, but by 16 h after injection the rate of excretion of ^{14}C in the urine had decreased to a much lower rate that was also nearly linear with time. The low rate persisted for up to 48 h after injection in the one subject who received each of the labeled atropines on separate days.

Kalser and McLain (105) found that glucuronidation was an important metabolic pathway in the early excretion of atropine from the body, but that this type of reaction seemed not to be particularly important after 4 h from the time of injection of atropine. Referring to the work of Phillipson et al. (98), one may suppose that glucuronidation occurs when noratropine is produced by oxidative dealkylation of the nitrogen atom of the tropine moiety of atropine. The paper by Kalser and McLain indicated that excretion of $^{14}CO_2$ in the expired air reached a peak about 75 min after the intramuscular injection of atropine and suggested that by 4 h after such a dose it may have been fairly low again. Possibly, therefore, glucuronidation and oxidative dealkylation proceed contemporaneously and in a coupled fashion.

The two labeled atropines used by Kalser and McLain (105) yielded the same chromatographically distinguishable ^{14}C-containing substances in the urine excreted by their subjects. There were four principal peaks of radioactivity, all of which appeared to consist, at least in part, of glucuronides at the early times of collection of urine. Peaks of radioactivity with Rfs similar to those of labeled atropine and tropine were the most prominent ones in the paper chromatograms of the urine of these subjects. In samples of urine collected during the first 6 h after a dose of labeled atropine, a component of Rf 0.70 seemed to be converted to one of Rf 0.55 by incubation with beta-glucuronidase from beef liver.

In summarizing this work on the metabolism of atropine by man, Kalser (106) used Tonnesen's data (89) on the urinary excretion of atropine by five subjects to show that there is a fairly broad range of rates of excretion of atropine; during the 12 h after ingestion of atropine, these five subjects excreted 15.5-42% of the ingested dose in their urine. It is not surprising, therefore, that one subject given labeled atropine intramuscularly excreted only 35.3% of the injected dose during the first 8 h, whereas the five experiments reported by Kalser and McLain (105) yielded cumulative excretion of injected atropine in urine during the same period between about 63% and 112% of the injected amount. After omission of the highest value, which came from urine samples that the investigators thought might have been handled improperly, there is still a variation between 63% and 83%. (Three experiments with three different subjects yielded closely similar excretion rates, with a mean of about 80% of the injected dose. A fourth experiment with one of the same subjects yielded the low value of 63%.)

When the data on ^{14}C still in the body at various times after intramuscular injection of tropine-labeled atropines were plotted and analyzed to yield two linear regressions in time, the derived half-times ranged between 1.3 and 2.1 h for the early parts of the curves and between 12.5 and 38.0 h for the later parts. When the data for the subject studied most extensively by Gosselin et al. (104) after intramuscular injection of tropine-labeled atropine were treated similarly, the half-times were 1.8 and 16.5 h. Apparently, the two types of labeling led to very similar estimates of the rate of removal of atropine from the body.

Two other studies provide somewhat similar findings. In one (107), coma-producing doses of atropine sulfate were injected intravenously, $[^3H]$atropine being mixed with unlabeled atropine to permit a dose of 100 uCi of ^3H to be included in the total dose of atropine sulfate. One psychopath, one epileptic, and 13 schizophrenics were studied. Graphs of data from three patients were presented in the paper. One man received a total dose of 70 mg of atropine sulfate (1.06 mg/kg), another man received 150 mg (2.8 mg/kg), and the third patient, a woman, received 270 mg (4.5 mg/kg). All injections were said to have been intravenous, but the curve for the concentration of the isotope in the blood of the woman is like that from a subcutaneous injection. During the 24 h after injection, the recoveries of the label in the urine, from the lowest dose to the highest, were 27.1%, 48.0%, and 81.1%. The concentrations of the isotope in the blood of the two men decreased rapidly during the first 15 min and more slowly thereafter; in the woman, the concentration increased to a maximum at about 15 min after the injection and then decreased moderately slowly. In all three patients, the concentration of the isotope in the blood became zero by or soon after 24 h after injection.

Hayden et al. (108) used a radioimmunoassay method to measure the removal of atropine from human plasma in four subjects given intravenous injections of 0.32 mg of atropine. They found a very rapid decrease in the plasma concentration during the first 5 min after injection, followed by a slower decrease. By 4 h after injection, the atropine concentration in the plasma was only about 2.25% of that present 5 min after injection. The initial period of rapidly changing concentration of atropine in the plasma may be a period of mixing in the plasma and equilibration between the plasma and various tissues.

A paper by Virtanen et al. (109) presented graphs of concentration of atropine in the serum of two women given 1.3 mg of atropine intravenously. There was an initial very rapid decrease in concentration, estimated by a radioimmunoassay method, followed by a slower decrease. The half-times for removal of atropine from the blood in these two women were 2.09 and 1.86 h. These times are similar to those calculated by Kalser and McLain (105) for the early parts of their curves. The half-times in the paper by Gaszner et al. (107) correspond more nearly with those calculated by Kalser and McLain for the later parts of their curves.

Because tropic acid was the only substance found after alkaline hydrolysis of urine from subjects given tropate-labeled atropine and a substance that was either tropine or a close relative was the only one found in large quantities after alkaline hydrolysis of urine from subjects given tropine-labeled atropine, it is apparent that atropine did not undergo drastic metabolic modification in human subjects. Oxidative dealkylation, with excretion of $^{14}CO_2$ in the expired air, probably led to the temporary formation of noratropine. This compound may then have been glucuronidated directly or been oxidized further to the hydroxylamine form and then glucuronidated. The finding that glucuronidation became a relatively unimportant reaction within about 4-6 h after the injection of atropine into muscle suggests that there is some sort of coupling between oxidative dealkylation of the tropine moiety of atropine and glucuronidation; in other words, glucuronidation may depend on the presence of some particular concentration of substrate, whether that substrate be noratropine or hydroxynoratropine.

HUMAN DATA FROM THE GENERAL LITERATURE

Gordon and Frye (27) collected from the literature reports on 11 deaths of humans considered to be due to atropine. Five of these involved application of solutions of atropine to the eyes--one person was thought to have received 1.6 mg of atropine and another 18.1 mg.

Four deaths were related to ingestion of atropine. One person had taken 198 mg of atropine with 100 mg of morphine. A 3.5-yr-old child had taken 540 mg of atropine and 1,350 mg of phenobarbital. Another person was reported to have taken a daily dose of tincture of belladonna equal to 1.2-2.5 mg of atropine for 6 yr before death (probably the highest dose for most of the time); there was nothing in the report to indicate clearly that death was attributable to long-term ingestion of atropine (110). Two deaths were connected with injection of atropine, in one case of 32 mg. On the other side of the picture, Alexander et al. (111) reported that an adult had survived after ingesting 1,000 mg of atropine; Mackenzie and Piggott (112) told of three children weighing 14-24 kg who lived after taking 600 mg of atropine.

Reports of cases of serious, nonfatal intoxication by tropic acid esters indicate that recovery from such episodes is reasonably rapid. For example, Sims (113) reported the case of a 67-yr-old woman who had applied belladonna plasters to her back for a month to relieve backache. During this time, the skin to which the plasters had been applied became excoriated. On the day of admission to a hospital, the patient had disturbed her husband in the early morning by wild and incoherent mutterings. On admission, she had two plasters on her back and was delirious and flushed. Her pupils were dilated equally and unreactive to light. Her arms were held in flexion and the legs in extension. After removal of the plasters from her back, she regained consciousness within about 12 h. Her pupils remained dilated for more than 36 h, however. She was released from the hospital as fully recovered, including healing of her skin, on the tenth day after admission.

Intoxication by scopolamine with similar signs was reported by Beach et al. (114). A 35-yr-old woman had taken 12 Sominex tablets (equivalent to 3 mg of scopolamine). She was in stupor with occasional clonic convulsions. Her pupils were dilated, her mouth was dry, her skin was hot to touch, her pulse was rapid, and she reacted vigorously to minor stimuli. When she was put to bed, her hands and fingers made repetitive plucking movements at the bedsheet. Apparent visual hallucinations, evidenced by avoidance movements, accompanied by screaming and severe disorientation began to abate after 12 h. The patient was discharged on the fourth day after admission to a hospital.

Stoll (26) compared the actions of atropine and methylatropinium nitrate on several functions of the human body. Methylatropinium nitrate at 10 µg/kg produced mean increases (four subjects) in pulse rate twice those produced by the same dose of atropine. In a series of experiments in which subjects were given identical doses (20 µg/kg) of the two drugs, they were found to have almost identical effects on pulse rate. Methylatropinium nitrate induced somewhat greater increases in the mean systolic and mean diastolic blood pressures and a considerably greater increase in the minute volume of the heart than atropine. Atropine increased the consumption of oxygen, whereas methylatropinium nitrate decreased it. Methylatropinium nitrate was about twice as effective as atropine in increasing skin temperature.

Cullumbine et al. (115) studied the effects of atropine sulfate on healthy men. Twenty men each received 1 mg of atropine sulfate by subcutaneous and intramuscular injection and 0.5 mg by intravenous injection. Forty men received double doses by the same three routes of administration. The first sign of action by atropine in many subjects was a slight, temporary decrease in heart rate, followed by a gradual increase. Intravenous injection produced effects more rapidly than the other routes of administration. The increase in heart rate induced by 1 mg of atropine sulfate injected intravenously was similar to, but of shorter duration than, that induced by subcutaneous or intramuscular injection of 2 mg. Subcutaneous injection of atropine sulfate induced acceleration of the pulse more rapidly, but less lastingly, than the same dose injected intramuscularly.

All of 44 men given 5 mg of atropine sulfate complained of dryness of the mouth and throat, of dizziness, giddiness, or light-headedness, and of difficulty in reading. Other complaints, in order of diminishing frequency, were of difficult urination; of tiredness, lassitude, or sleepiness; of headache or eyeache; and of nausea. After 3 mg of this drug, the only complaint voiced by 12 men so treated was of dryness of the mouth and throat; after 2 mg of atropine sulfate, only 37 of 45 subjects complained of dryness of the mouth and throat. The most definite objective effect of atropine was an increase in heart rate. Systolic blood pressure tended to decrease slightly and diastolic pressure to increase. There were also slight, but insignificant, changes in the concentrations of hemoglobin, leukocytes, and blood glucose. Some subjects had changes in their electrocardiograms that indicated sinus arrhythmia, P-wave enlargement, premature atrial contractions, and flattened, coved, or inverted T waves. Daily injections of 2 mg of atropine repeated for 5 d decreased either the severity of or the subjective response to the changes in secretion by the glands of the mouth and in accommodation of the eyes to light and to nearness.

Wyant and Dobkin (116) compared atropine, L-hyoscyamine, and scopolamine for ability to stop salivation induced by intravenous injection of a mixture of carbachol and epinephrine. Scopolamine was slightly more active in this regard than L-hyoscyamine, and both were considerably more active than atropine.

Bachrach (117) surveyed the literature related to a group of anticholinergic drugs, including atropine and methylscopolammonium bromide, and reported the results of studies of small groups of patients. Atropine was about 4 times as active a sialolytic drug as methylscopolammonium bromide, but produced more side effects. In a group of 11 subjects given 0.6 mg of atropine orally, nine complained of side effects; blurred vision was the most common of these; one subject complained of dysuria, and another of nervousness, dizziness, and anorexia. Only one of eight patients given about 2.4 mg of methylscopolammonium bromide complained of blurred vision, and one complained of dysphagia.

Herxheimer (118) compared a small group of anticholinergic drugs--including atropine sulfate, scopolamine hydrobromide, and methylscopolammonium bromide--for ability to affect several functional systems of man after subcutaneous injection. Scopolamine hydrobromide (0.2-0.8 mg) was strikingly more active than either methylscopolammonium bromide (0.125-1.0 mg) or atropine (0.5-2.0 mg) in inducing dilatation of the pupil and paralysis of accommodation. Methylscopolammonium bromide was more potent than scopolamine hydrobromide, which was more potent than atropine, in inhibiting secretion of saliva. The two salts of scopolamine were equally potent, and more so than atropine, in slowing urination. Methylscopolammonium bromide was strikingly more potent than atropine sulfate in increasing heart rate. The incidence of drowsiness was 100% with scopolamine hydrobromide, about 25% with methylscopolammonium bromide, and lowest with atropine. The effects of methylscopolammonium bromide and atropine sulfate on heart rate became evident (18-19 min) and reached their greatest intensities (37-40 min) at about the same times, despite the fact that the dose of methylscopolammonium bromide was only 22.5% that of atropine sulfate. When equally effective doses of the compounds were administered, methylscopolammonium bromide was slower than scopolamine hydrobromide and atropine sulfate in exerting its maximal effect on salivation and on the iris. The effects of scopolamine hydrobromide on the iris and on accommodation for near vision lasted considerably longer than those of equal doses of methylscopolammonium bromide and atropine sulfate.

Using salivary secretion as the indicator, Mirakhur (118) found that atropine and scopolamine were about 2 and 4-5 times as active, respectively, after intramuscular injection as after ingestion. When heart rate was used as the indicator, both alkaloids were about twice as active after intramuscular injection as after ingestion. When the secretion of sweat was used as the indicator, however, ingested scopolamine was nearly as active as that injected intramuscularly, whereas atropine was about twice as active when ingested as when injected.

In a combination of literature review and reports of personal experiences with patients, Schweitzer and Mark (120) summarized the effects of atropine on the intracardiac conduction system and the myocardium. The chronotropic effects of atropine are the best-known ones on the heart, consisting of an early and brief (a few minutes) slowing of the heart, most apparent with small doses or with doses administered by routes likely to yield slow entrance of the substance into the body, and a later and predominant increase in heart rate. The predominant action is due largely to blockade of vagal impulses to the heart, but is contributed to by improved sinoatrial conduction, increased frequency of discharges by the atrioventricular node, and facilitation of conduction through that node. Atropine may affect the refractory period of atrial muscle and conduction by the His-Tawara bundle, but these actions are uncertain. Atropine may induce atrial fibrillation, atrial ventricular dissociation, ventricular tachycardia, and even ventricular fibrillation. The last result is particularly likely (122,123) when the work of the heart is increased suddenly under conditions of hypoxemia, as when atropine is administered intravenously to an apneic person who has just been overcome by exposure to an inhibitor of cholinesterases and whose heart still beats.

Despite the reputation of scopolamine for causing amnesia, Hardy and Wakely (124) found that only 13 of 100 patients given subcutaneous injections of hyoscine at about 6.3 µg/kg and morphine at about 157.2 µg/kg had any amnesia of being shown a card just before induction of anesthesia. Only seven of these patients had complete amnesia of preoperative events. When atropine at about 9.4 µg/kg was substituted for scopolamine in the preanesthetic mixture, only one of 100 patients had partial amnesia of being shown a card before induction of anesthesia.

Dervent and Karacan (125) examined the effects of intramuscular injections of atropine sulfate (15-60 µg/kg), scopolamine hydrobromide (1.5-6 µg/kg), and methylscopolammonium bromide (1.5-6 µg/kg) on rapid-eye-movement (REM) sleep and its neurogenic correlate, nocturnal penile tumescence (NPT). The drugs were injected 2 h after onset of sleep. Fifteen healthy male volunteers between the ages of 21 and 35 were used. Scopolamine at 6 µg/kg and atropine at 60 µg/kg suppressed both REM sleep and NPT. Quaternized scopolamine had neither of these effects.

Ostfeld et al. (126) gave oral doses of 10 mg of atropine sulfate in 100 ml of water to 10 subjects and recorded the rate of salivation, the grades in the seven moods of the Clyde Mood Scale, the electroencephalogram, and EEG arousal induced by single and repetitive flashes of light before and after drug administration. Atropine increased heart rate by 60% and decreased the amount of saliva produced during 1 min by 68%. Five subjects complained of mild difficulty in urinating. These effects were maximal at 2 h after atropine was ingested, as were those on EEG arousal by flashes of light. The last effects consisted of decreasing the duration of the arousal induced by single flashes (by 60.6%) and repeated flashes (by 57.1%). There was a slight tendency for arousal by repeated flashes of light to be affected before that by single flashes. In the spontaneous EEG recordings, waves of lower amplitude and longer duration (5-8 per second) replaced the normal waves at 8-12 per second, beginning at about 30-60 min after atropine ingestion. Observation of spontaneous behavior and evaluation of mental status and mood revealed that atropine shifted behavior in the direction of reduced energy, decreased voluntary speech and motion, reduced attention span, impaired memory of contemporaneous events, and drowsiness. The changes in the spontaneous EEG and in behavior appeared at about the same time.

Ostfeld and Aruguete (127) performed a similar study in which 54 volunteers were subjected to subcutaneous injections of 150-800 ug of scopolamine hydrobromide. The lowest dose induced moderate bradycardia, but decreased salivation. The highest dose completely blocked the secretion of saliva and seemed to induce sleep, hallucination, and mental disorientation more frequently than the dose of 10 mg of atropine sulfate used by Ostfeld et al. (126). The larger doses were associated with decreased ability to perform tasks requiring close attention.

Commin et al. (128) reported that intravenous injection of physostigmine sulfate at 40 µg/kg into six patients in coma as a result of administration of atropine was capable of decreasing the depth of the coma, but that the duration and extent of the change were variable. The treatment could be repeated, however. In a later paper, the same group of investigators (129) reported that treatment with physostigmine in six cases of attempted suicide with anticholinergic preparations could be monitored with EEG recordings, using EEG alerting as a signal of effect. When the preparations used included overdoses of drugs other than atropine or other anticholinergic substances, physostigmine had no effect. They pointed out that therapy for anticholinergic coma with physostigmine was limited by the short period of its activity and by its secondary effects, including premature ventricular contractions and convulsant activity.

Hollender et al. (130) reported on a woman who complained of auditory and visual hallucinations after taking two Sominex tablets. It developed that she had also taken chlorpromazine, which has distinct anticholinergic activity, and amitriptyline, which has the greatest anticholinergic activity of any of the common tricyclic antidepressants. The woman's pupils were markedly dilated and fixed and did not constrict when drops of a 1% solution of pilocarpine were applied to the eyes. Eventually, she was found to have been using eyedrops of tropicamide, a potent but nonpersistent mydriatic. Shortly after admission to the hospital, the woman had a seizure. This was thought to have been of psychogenic origin; there were no recurrences. With no drug treatment and prevention of any access to drugs by the patient, her disorientation cleared rapidly. (She claimed, however, that hallucinations persisted during her stay in the hospital of 2 wk, but the investigators considered that she was probably attempting to prolong her hospitalization.)

Aucamp and Meyer (131) reported that well-controlled epileptics may experience sudden exacerbation of their seizures if they begin taking drugs with anticholinergic activity (as in some antacid preparations) or antihistaminic activity (as in preparations for amelioration of colds and influenza).

Moskovitz et al. (132) analyzed the hallucinations experienced by a group of 88 patients, 32-84 yr old, with idiopathic parkinsonism for at least 6 mo. The therapeutic regimens when these patients began to hallucinate had included anticholinergic drugs with or without amantadine (13.9%), alone (23.3%), and with amantadine or anticholinergic drugs or both (62.8%). The hallucinations had been visual for 87.1% of the patients, auditory for 35.5%, and tactile for 4.8%; 25.8% had both visual and auditory hallucinations. The hallucinations were nonthreatening for 72.7% of the patients and threatening for 27.3%. The investigators believed that anticholinergic drugs probably were the principal factors in giving rise to hallucination.

Holland et al. (133,134) gave men intramuscular injections of sterile water, 2 mg of atropine sulfate with 500 mg of N-methyl-2-hydroxyiminomethylpyridinium methyl sulfonate (P2S), 750 mg of P2S, 750 mg of P2S with 2 mg of atropine sulfate, or 2 mg of atropine sulfate. The initial rate of absorption was judged by the time after injection required for attainment of the maximal reduction in heart rate. Five volunteers were used to examine the effects of water, 750 mg of P2S, 750 mg of P2S with atropine sulfate, and atropine sulfate alone; seven were used to determine the effects of 750 mg of P2S with atropine sulfate and atropine sulfate alone; 10 were used to compare the effects of 500 mg of P2S with atropine sulfate and atropine sulfate alone.

EEGs were recorded after the subjects had reclined quietly on beds for an hour. The injection was then given into the upper outer aspect of the thigh, and ECG recording continued for about 2 hr. The air temperature was 21-24°C. All 22 volunteers had control ECGs recorded on day 1. After 2 or 3 d, volunteers 1 to 12 were given 2 mg of atropine sulfate; 5 d later, they received a mixture of 2 mg of atropine sulfate and 750 mg of P2S. Five of these volunteers were given sterile water 2 d after the injection of the mixture of atropine and oxime; a day later, these five received 750 mg of P2S. The 10 remaining volunteers received injections of 2 mg of atropine sulfate and 5 d later of 2 mg of atropine sulfate and 500 mg of P2S.

The heart rates of the volunteers given no injection, injections of sterile water, or injections of 750 mg of P2S had closely similar variations with time. The graphs of heart rate after injections of 2 mg of atropine sulfate, of 2 mg of atropine sulfate and 500 mg of P2S, and of 2 mg of atropine sulfate and 750 mg of P2S followed closely similar courses with time after injection. These were distinctly different from the first set of lines. One can conclude that 750 mg of P2S alone had no significant effect on heart rate and that addition of either 500 or 750 mg of P2S to 2 mg of atropine sulfate had no striking effect on absorption of atropine from a site of intramuscular injection. The initial rate of absorption of atropine seems to have been increased slightly by both doses of P2S (mean time to maximal bradycardia was decreased from 18.7 min to 12.9 min by 500 mg of P2S and from 15.3 min to 12.2 min by 750 mg of P2S).

Martin (135) followed up this work by comparing the effects of intramuscular injections of 2 mg of atropine sulfate alone and mixed with 750 mg of P2S on heart rate and sweating in nine exercising men. These volunteers worked at 25°C and a relative humidity of 65-75% until their heart rates had risen to 110-130 beats/min and then got injections of atropine alone or of atropine and P2S. The exercise was continued for an additional period. The dose of atropine increased the mean heart rate at the end of the period of exercise by 34.4% and decreased the mean rate of sweat production by 36.1%. Addition of P2S to atropine had no important effect on these changes.

The General Practitioner Research Group (136) surveyed the effects of drugs in women in the United Kingdom during the first trimester of pregnancy. Of 661 pregnant women who took drugs of some sort during the first trimester, 57 (8.6%) had either miscarriages, stillbirths, neonatal deaths, or abnormal children. Antihistamines were involved in 38 of these, barbiturates in 10, and female sex hormones in 10. Antihistamines had been used by 76.9% of the pregnant women, barbiturates by 9.2%, and female sex hormones by 9.1%. Thus, women who used barbiturates or female sex hormones during the first trimester of pregnancy were the most likely to have one of the four types of reproductive accidents considered. A pregnancy that resulted in a blind infant had involved the use of scopolamine during the eighth week and of promethazine during the ninth week. Scopolamine was the only tropic acid ester used by a pregnant woman who delivered an abnormal child in this survey.

Hellman *et al.* (137) studied the reliability of the response of the fetal heart to administration of atropine to a pregnant woman, the fetal heartbeat being recorded and analyzed by a computer as an indicator of transplacental transfer. Intravenous injection of atropine sulfate at 22 ug/kg during a 2-min period to 28 normal pregnant women resulted first in slowing of fetal heart rate and then tachycardia in 26 cases. In two cases, there was no apparent transfer of atropine to the fetus, although both women delivered apparently normal infants.

Mellin (138) studied the consequences of the use of drugs during the first trimester of pregnancy by 3,200 pregnant women seen at the Columbia-Presbyterian Medical Center in New York during 1953-1957. Of these 3,200 pregnancies, 266 were recorded as resulting in malformed infants. Two control groups were established by selecting 266 records immediately preceding and 266 immediately after each record relating to a malformed infant. Drugs were used during the first trimester by 53% of the women who delivered malformed babies, whereas 50.9% ± 1.9% of the women in the control groups had used drugs during the first trimester. The difference is not significant. However, when fetal and neonatal deaths among the offspring of women who used drugs during the first trimester and of those who did not were summed separately, only 4.9% of the offspring of the latter group died in utero or soon after birth, whereas 9.5% of the offspring of the drug-users died before or soon after birth. Atropine was found to have been taken by one woman whose infant died soon after birth among 13 offspring of women who had used this drug during the critical period of embryogenesis; three of the other 12 babies of these women were malformed. Of three women who had taken scopolamine during the first trimester, two delivered apparently normal children. There is no way of determining from the paper what other drugs the women who used these two anticholinergic substances may have taken, except that one of seven babies with both congenital heart disease and another unspecified malformation was born to a woman who had used chlorpromazine, dimenhydrinate, meperidine, morphine, and secobarbital sodium in addition to scopolamine.

Hellman and Fillisti (139) extended the study by Hellman _et al._ (137) of placental transmission of atropine in normal women by studying 24 pre-eclamptic women, three with eclampsia, 11 with chronic hypertension, and 16 with diabetes. Sixteen of the 24 pre-eclamptic patients, two of the three eclamptic patients, and four of the 24 records on the 16 diabetic women gave no evidence of transfer of atropine to the fetus. Two of the negative records on diabetic mothers can be paired with one each from the same woman that provided evidence of transplacental transfer of atropine. Another of the negative records pertained to a woman who had yielded three records indicating placental transmission of atropine to the fetus. The general conclusion from both studies (137,139) was that the atropine test of placental function is not sufficiently precise to indicate whether placental function is normal in any given case.

Quilligan (140) pointed out that comparison of blood from the umbilical veins and arteries of placentas of six women who received 1.0-1.5 mg of atropine and of 13 women who received no atropine revealed that the umbilical blood from the atropine-treated women had lower pO_2s than that from the control patients, although the acid-base relationships of the umbilical blood from the two groups were not particularly different.

Kivalo and Saarikoski (141) studied transplacental transmission of atropine by injecting [^{3}H]atropine intravenously (0.5 µg/kg) into 25 women undergoing delivery by Cesarean section. Samples of blood were taken from the antecubital vein of the mother and from the umbilical vein and artery. The investigators found that the concentration of the label in blood from the umbilical artery was about 50% that in blood from the umbilical vein. At 1 and 5 min after injection, the concentrations of ^{3}H in the blood from the umbilical vein were 12% and 93%, respectively, of those in the maternal venous blood at the same times. Atropine seems to cross the placenta rapidly.

Onnen et al. (142) estimated the concentrations of atropine in blood samples by bioassay on isolated ileum from guinea pigs stimulated by acetylcholine. They gave rapid intravenous injections of atropine sulfate to 28 pregnant women at 12.5 µg/kg and found that tachycardia in the fetus started 5-10 min after injection, at a time when the initial rapid decrease in the atropine concentration in the mother's blood had been replaced by a comparatively low rate of decrease. In another experiment, they gave 45 women in labor atropine injections and took samples of maternal and of mixed cord blood at birth. They found that 5-15 min after injection the ratio of the mean concentration of atropine in cord blood to that in maternal blood was 1.2 ± 0.5. This paper also indicates that atropine is transferred across the placenta within a matter of a few minutes.

In addition to the effects on the reproductive functions of the female, there has been some study of the effects of tropic acid esters and related compounds on sexual function in the male. One paper on this topic (125) has been mentioned above. Horowitz and Goble (143) have reviewed the literature on drug-induced sexual dysfunction in the male and have concluded that any drug with atropine-like effects may interfere with penile erection. (They pointed out that impotence induced by antimuscarinic drugs may occur without reduction of libido and may thus be particularly frustrating to the male.)

Knuutila et al. (144) reported finding an unusually high incidence of chromosomal aberrations (broken chromatids, acentric fragments, dicentric chromosomes, etc.) in lymphocytes from a woman who had been vaccinated against rubella and 3 d later had received a variety of drugs, including atropine, in connection with an operation on varicose veins; after the operation, she had been given indomethacin for 10 d and furosemide for 12 d. The young woman was considered to be in good health. Lymphocytes cultured from the blood of four other women who had been vaccinated against rubella also contained unusually large numbers of broken chromatids, but did not have the other chromosomal abnormalities. The percentages of metaphase cells with broken chromosomes from these four women were less (by 3-11%) than that in the blood from the first patient (15%). The investigators suggested that the major aberrations in the first patient's chromosomes may have resulted from a combined effect of the vaccine and one or more of the 10 drugs that she took during and after her operation. They recommended, therefore, that prescribing drugs should be avoided, if possible, near the time of vaccination for rubella. (In view of the fact that the investigators had no knowledge of the state of the patient's chromosomes before her vaccination and operation, their suggestion and recommendation seem to be on rather shaky ground.)

Minich _et al_. (145) attempted to detect mutagenic products in the urine of patients who were taking a large variety of drugs, including atropine, as a part of their therapeutic regimens in the hospital. The urine was tested with and without the addition of a preparation of microsomes from the livers of rats that had been given a 1% solution of phenobarbital in place of drinking water for 6 d before removal of the livers. Minich _et al_. found no evidence of mutagens in the urine of the patients given atropine.

HUMAN DATA FROM EDGEWOOD ARSENAL

Grob _et al_. (147) compared the effects of atropine administered intravenously and intramuscularly. Four subjects were used. Two received injections of 2 mg of atropine sulfate by the intramuscular route only; one of these subjects received only one injection, and the other, two. A third subject received 1 mg of atropine sulfate intravenously on one occasion, 1 mg of atropine sulfate intramuscularly on another occasion, and 2 mg of atropine sulfate intramuscularly on four other occasions. The fourth subject received 1 mg of atropine sulfate intravenously on two occasions and 2 mg of atropine sulfate intramuscularly on three occasions. Atropine sulfate by either route of administration induced an increase in heart rate, an increase in skin resistance, an increase in pupil size, dryness of the skin, and an increased sensation of dryness of the mouth. In general, the effects appeared earlier after intravenous than after intramuscular injection. Prior administration of sufficient TEPP to increase sweating and salivation and to induce anorexia and mild nausea slightly delayed the onset of the effects of atropine and slightly reduced the extent of those effects.

Marzulli and Cope (148) gave 40 men single injections of 1 mg of atropine intramuscularly, 28 men two doses of 1 mg of atropine 90 min apart, and 20 men single doses of 3 mg of atropine. The last group of subjects contained men who had received the two doses of 1 mg of atropine at least 2 wk previously and the single dose of 1 mg still earlier. The injections were into a deltoid muscle. Testing of the subjects began 30 min after each dose of 1 mg of atropine and 60 min after the dose of 3 mg. A battery of 10 tests, including nine for grading visual acuity, stereopsis, and other properties of the eye and one for grading cognition, was used.

Four of 25 subjects who had received intramuscular injections of saline reported slight dryness of the mouth, two complained of headache, and one who had complained of headache also mentioned a slight numbness of the lips.

The 40 men given intramuscular injections of 1 mg of atropine all reported dryness of the mouth; 17 complained of dizziness, 13 of feeling tired and sleepy, 10 of dryness of the nose, and eight of having difficulty in reading.

The 28 men who received 2 mg of atropine all complained of dryness of the mouth; 17 complained of feeling tired and sleepy; 12 reported either that they had slept during the day or had gone to bed earlier than usual; nine complained of feeling incoordinated.

The 20 subjects given 3 mg of atropine all reported dryness of the mouth; 18 had felt tired or sleepy; 14 recalled either that they had slept during the day or had retired early in the evening; seven reported that their sleep during the night after the injection had been broken by vivid and disturbing dreams. Three men in the latter group reported seeing objects in unusual colors; a fourth had a visual aura. No true hallucinations were described.

The only significant objective changes seen in this study were a decrease in the ability of the eye to accommodate for near vision and tachycardia. The mean magnitude of accommodation by the eyes of the 20 men before they were exposed to atropine was 10.3 diopters (148). After a single dose of 1 mg, the mean magnitude of accommodation was 8.5 diopters. When the same subjects had been given two doses of 1 mg, the mean amount of dioptric change was 7.5. After a single dose of 3 mg, the eyes of the same 20 subjects had a mean magnitude of accommodation of 4.8 diopters.

This report provided no data to enable the reader to judge whether the tachycardia that followed the various doses of atropine was dose-related; data on heart rate are provided only after the single dose of 3 mg. The mean heart rate in the 20 men before exposure to atropine was 75.8 beats/min. At 15 min after injection, the mean was 116.8 beats/min. After another 15 min, the mean was 117.8 beats/min. Thereafter, the heart rate decreased gradually, the mean at 7-8 h after the injection being 66.1 beats/min. The mean was below that during the control period from the third hour to the eighth hour after injection.

Himwich (149) used four subjects to study the effects of intramuscular atropine at 0.07-0.12 mg/kg on the EEG, personality structure, heart rate, breathing rate, and pupil diameter. The pupil diameter increased by a mean of 114.3%, the heart rate by a mean of 51.0%, and the breathing rate by a mean (for only three subjects) of 48.5%. There was an increase in the voltage of the predominant frequency in the EEG, impairment of the alerting reaction on opening the eyes, and increased proportions of slow waves and spindles in the EEG, even though the subjects were awake and responsive to spoken commands. All four subjects found concentration on a single topic difficult, both attention and logical progression of thought being attenuated. Judgment became poor, sensitivity to the environment was lessened, the grasp of reality was weakened, and drowsiness affected all four subjects. Three of the subjects became irritable, although the mood of the fourth was predominantly euphoric. The three irritable subjects had disturbed sleep and fantastic dreams during the night, whereas the fourth slept well. All four subjects had some degree of ataxia; two complained of urgency of urination and congestion of the conjunctivae. The most persistent effect of atropine noticed in this study was paralysis of accommodation, which disappeared gradually during 3-4 d after injection.

Wechsler and Koskoff (150) used nine surgical patients to study the effects of doses of atropine between 1.3 and 5.85 mg--administered by intravenous, intramuscular, or intracisternal injection--on cerebral blood flow and metabolism and on neural and cardiac indexes of normal function. One patient received atropine intracisternally on two occasions, 1.8 mg the first time and 4.2 mg the second time. Three patients were given intravenous doses of 1.3-1.6 mg of atropine, and five were given intramuscular doses of 2.0-5.85 mg. The intracisternal injections induced no changes in heart rate or blood pressure within 30 min after injection. The intravenous injections produced tachycardia in the three patients so treated and small increases in mean arterial blood pressure in two of them, whereas the third had a small decrease in blood pressure within 3 min after injection. All five patients given intramuscular atropine developed tachycardia, and four of them (the only ones whose blood pressures were recorded) developed small increases in mean arterial blood pressure. Atropine by any route of administration in these studies induced no consistent changes in cerebral blood flow, cerebral vascular resistance, cerebral consumption of oxygen, cerebral arteriovenous oxygen difference, cerebral respiratory quotient, or pH of blood from the jugular vein. The only significant neurologic change after administration of atropine was dilatation of the pupil. Only one patient given atropine yielded any indication of an effect on the EEG: a minimal depression of the alpha component. No morbidity was attributable to the atropine. The authors concluded that timidity in the use of atropine is not warranted.

Andrews *et al*. (151) used nine subjects in a preliminary comparison of the effects of atropine sulfate injected intramuscularly and subcutaneously in warm (35°C, 80% relative humidity) and cool (16°C, ambient relative humidity) environments and 24 subjects in a preliminary comparison of the effects of inhalation of 2 mg of atropine sulfate as a dust and as an aerosolized solution in water. Finally, 10 subjects were used to repeat the comparison of the effects of atropine sulfate administered by the two methods of injection in warm and cool environments and to compare the effects of 2 and 5 mg of atropine sulfate inhaled as a dust and an aerosolized aqueous solution in the warm environment. The report did not state whether the same subjects were used in more than one part of this study.

The results of the study indicated that 2 mg of atropine sulfate had practically identical effects on heart rate and sweating, whether injected intramuscularly or subcutaneously.

The mean increase in heart rate after injection of 2 mg of atropine sulfate in the warm environment was 42.7%, whereas that in the cool environment was 31.3%. The mean increase in heart rate after inhalation of 2 mg of atropine sulfate as a dust or an aerosolized solution was 23.7%; the mean increase after inhalation of 5 mg of the drug was 48.1%. The increases with the two types of dispersion of atropine sulfate were essentially identical for the two doses used. It is apparent that inhalation of atropine sulfate is not as effective a method of delivery, with heart rate as the indicator, as intramuscular or subcutaneous injection.

Although inhalations of 5 mg of atropine sulfate induced an increase in heart rate comparable with that induced by the injection of 2 mg, all subjects reported headaches, giddiness, tiredness, lethargy, and muscular weakness or incoordination after inhaling the 5-mg doses. It took 24 h for all the subjects to feel able to return to their usual activities after inhaling the larger dose of atropine sulfate, and several reported visual hallucinations, euphoria, and dysuria. The authors concluded that the use of inhalators for self-administration of atropine sulfate would be impractical and that intramuscular injection would be the easiest method to teach to nonmedical persons.

White et al. (152) gave 12 subjects intramuscular doses of atropine sulfate at 22.6-134.0 ug/kg (mean, 50.8 μg/kg). The mean blood pressure before administration of atropine sulfate was 123/79; after administration, it was 129/91. The mean rate of the radial pulse was 69 beats/min before administration of atropine; within 30-60 s after injection, it was 61.5 beats/min; it increased later, reaching a mean maximum of 100.5 beats/min. The mean breathing rate before atropine was administered was 15.1/min; after atropine had been administered, it rose to 17.1/min. The mean diameter of the pupil was estimated to be 4.8 mm before administration of atropine; under the influence of that drug, it increased to 6.5 mm. Eight of the 12 subjects had extensions of the near point under the influence of atropine, the subjects given the two largest doses having the greatest increases.

All 12 subjects complained of dryness of the mouth; six became restless; two complained of sensations of heat on their skins, although they were in an air-conditioned room; 10 were observed to become drowsy; 10 found that walking a straight line was difficult; and five had malaise, headache, vertigo, and slight nausea. These complaints had disappeared by the following morning.

Other effects of atropine sulfate seen in the 12 subjects who took part in this experiment, in order of decreasing incidence, were sleepiness, shortened attention span and blurred vision, dreaming, eye irritation, lack of desire for mental work, and urinary urgency. The usual changes in the EEG pattern after administration of atropine were a flattening of the record, a decrease in frequency, and intermittent blocking of the alpha rhythm. There was a decrease in alerting on opening the eyes. Five subjects had notable improvement in photic driving after administration of atropine, and five others had slight improvement. Only one subject had a decrease in response to photic stimulation.

In attempting to devise a battery of tests useful in assessing the effects of chemicals on human capabilities, Elkin et al. (153) gave 11 subjects intramuscular injections of scopolamine at 12 µg/kg after the subjects had spent a day gaining familiarity with the test battery. Four subjects received placebo injections. The only part of the test battery that may have been affected by the placebo was a manual-dexterity test in which the subject moved as many blocks as possible in 30 s from one hole to an adjoining hole. The number of blocks moved 0.75 and 2.25 h after the placebo was about 8.3% greater than the number moved before administration of the placebo and 4.25 h after the placebo. Scopolamine decreased both near and far visual acuities, the effect on near visual acuity being much the greater. In the manual-dexterity test, scopolamine decreased by 38.6% the number of blocks moved, this decrease persisting for 2.25 h after the injection of scopolamine and then diminishing gradually. By 8.75 h after the injection, the number of blocks moved was only slightly below the baseline measurement. Mean grip strength was lowered from about 54 kg to about 48 kg by the dose of scopolamine. Scopolamine lowered the mean duration of balancing on a beam on one foot from about 17 to about 5 s. The mean number of additions performed within 3 min decreased from about 42 to about four. The mean number of digits that could be recalled after auditory presentation decreased from about 7.1 to about 5.1. The mean ability to estimate 10 was altered only slightly by scopolamine in the direction of underestimation. However, the variance of this estimation was increased by about 128% by scopolamine. The mean reaction time (the subject pressed a switch as soon as possible after receiving a visual cue) increased from 0.22 to 0.38. The authors concluded that the most dramatic change was in near visual acuity. Grip strength, simple reaction time, and estimation of elapsed time were affected only slightly by scopolamine.

Kitzes et al. (154) analyzed the records of 18 subjects who had been given intramuscular injections of atropine at 32-125 ug/kg and who weighed 57.2-90.0 kg. The results on the Number Facility Test (completion of as many additions as possible during 3 min) were used to grade each subject's response to the drug. No correlation between body size and decrease in the Number Facility Test was found.

Cummings and Craig (155) exposed 10 men to temperatures up to about 52°C to induce a range of rates of sweating and then subjected them to a variety of doses of atropine sulfate by intravenous injection of various priming doses followed by infusions of various concentrations of the drug for 13-53 min. The total dose of atropine received by a subject varied between zero and 1.6 mg; the report was unclear on whether this value is for atropine alkaloid or atropine sulfate. Higher rates of sweating, induced by exposure to high environmental temperatures, were found to require larger doses of atropine to inhibit sweating. Sweating recovered rapidly (6-74 min) after the end of infusion of atropine sulfate, particularly at the higher temperatures.

Kligman and Copelan (156) reported that 13 subjects had been exposed to atropine. No details or results of the study were given.

Crowell and Ketchum (157) used 33 normal male volunteers to assess the ability of physostigmine to antagonize the deliriant activity of scopolamine. The volunteers received intramuscular injections of scopolamine hydrobromide at 24 µg/kg; subgroups of the intoxicated men received physostigmine salicylate intramuscularly at 50 ug/kg 15, 30, or 90 min after injection of scopolamine. Those treated 15 min after scopolamine did not have any immediate reversal of feelings of fatigue and drowsiness and gradually developed a typical delirium, but at a lower rate than those who had not been given physostigmine. Physostigmine lowered the heart rate in these men and maintained it on a plateau below that in the untreated group until the heart rate in the untreated group decreased to equal that in the treated group 3-4 h after the scopolamine had been administered. Physostigmine had only a minor and brief effect on the diameter of the pupil, but decreased significantly the decrement in the Number Facility Test due to the dose of scopolamine during the 2 h after its administration.

The subjects treated with physostigmine 30 min after the injection of scopolamine improved dramatically and rapidly in their performance of the Number Facility Test. These men relapsed into delirium about 3 h after administration of physostigmine and then recovered at about the same rate as the untreated men. The effects of physostigmine on heart rate and pupil diameter were similar to those in the group treated earlier in the intoxication.

The men treated 90 min after injection of scopolamine, after development of a full-blown intoxication, improved dramatically and rapidly, the change being noticeable within 10 min of injection of physostigmine and becoming maximal within 30 min. At that time, the subjects were alert, well-oriented, and logical. Their performance in the Number Facility Test decreased slightly about 1.5 h after the dose of physostigmine, but did not fall to near the level of performance of the untreated group at that time. The performances in the Number Facility Test of the untreated group and the group treated with physostigmine became essentially identical at about 6 h after the dose of scopolamine. The heart rate decreased within 30 min after the dose of physostigmine, but this drug had little effect on pupil size.

Baker et al. (158) summarized their studies with 82 volunteers given scopolamine. No details of the experiments or of their results were given, but the individual subjects were said to have reacted quite differently to a given dose of scopolamine. After extensive analysis of the data, the authors concluded that research was needed to identify the factors that cause "subject by treatment" interactions and that means for controlling for them were necessary for obtaining consistent effects by a given chemical.

Krieger (64) examined the effect of injected atropine on the circadian variation in the concentration of

17-hydroxysteroids in human plasma, using two healthy male subjects. One subject was given a subcutaneous injection of 3.0 mg of atropine, the other a similar injection of 3.5 mg. Three other subjects took oral doses of 2.5 mg of atropine. None of these subjects gave any evidence of an effect on the usual pattern of variation in plasma 17-hydroxysteroids. The author pointed out that the dose of atropine used in these human studies was only about one-tenth that used in experiments with cats in which atropine had been found to block the nocturnal rise in plasma 17-hydroxysteroid concentration, when administered before the time at which the circadian rise could be expected to occur.

Safer (159) investigated the possible interplay of sleep deprivation and scopolamine intoxication in producing deterioration of human abilities. Men were deprived of sleep for one or two nights and were then given scopolamine hydrobromide intravenously at 5 or 10 µg/kg. Behavior was rated with a checklist. The performances of the subjects in the Number Facility Test, a manual-dexterity test, and a vigilance test were evaluated. Eleven subjects were given scopolamine at 10 µg/kg, 10 were deprived of sleep for a night and given intravenous saline, six were deprived of sleep for a night and were given scopolamine at 10 µg/kg, two were given scopolamine at 5 µg/kg, four were given scopolamine at 5 µg/kg after being deprived of sleep for a night, and four were given scopolamine at 5 µg/kg after being deprived of sleep for two nights.

The combination of loss of sleep for one night and scopolamine at 10 µg/kg had a more than additive effect on performance in the Number Facility Test and considerably more effect on manual dexterity than the scopolamine alone. The combination also produced hallucinations in about 2.7 times as many subjects as the same dose of scopolamine alone. The results with the lower dose of scopolamine were similar to those reported above, but less striking. The men deprived of sleep for two nights became so somnolent after the dose of scopolamine that the tests could not be run.

The effects of large intramuscular doses of atropine on the electrocardiogram were examined by Hayes _et al_. (160, 161), with six healthy male volunteers. The volunteers were given atropine sulfate at 175 µg/kg. By 2 min after injection, there was slowing of the heart without any striking change in the ECG record. At 5 min after the dose, the Pwave was engulfed by the QRS complex, and an A-V nodal rhythm was instituted. This A-V dissociation was temporary, a sinus tachycardia becoming apparent at 6-7 min after the injection and persisting for about 3 h.

Four volunteers who had received scopolamine, three who had been given atropine, and nine who had received no chemicals were interviewed and examined at some unknown time after their service as experimental subjects (162). None of the subjects felt that he had suffered physical or psychic injury as a result of serving as a subject. Blood counts for all the former subjects were within the normal range. Three of the former control subjects and one former subject who had been given both scopolamine and a salt of LSD had up to 10 white cells per high-power field in their urine; that suggested low-grade urinary tract infection. One former subject who had been given atropine had a mildly positive test for urinary glucose (there are so many possible causes of such a situation that no precise meaning can be attached to it). One of the former control subjects had an increased activity of GOT in his serum, and another had a slightly increased concentration of BUN. No abnormalities of serum chemistry were recorded for the former subjects who had been given atropine or scopolamine. This study revealed, therefore, no definite evidence of long-term physical or psychic injury by esters of tropic acid.

Klapper et al. (163) examined the records of eight subjects who had been given intramuscular injections of atropine at 62 µg/kg, six who had been given atropine at 104 µg/kg, and six who had been given scopolamine at 11.8 µg/kg. The performance of each subject on the Number Facility Test after injection was compared with his scores on the three validity scales and 10 standard scales of the Minnesota Multiphasic Personality Index (MMPI). The best correlations between performance in the Number Facility Test and scores on scales in the MMPI for the men given atropine at 62 µg/kg were with those in the hypochondriasis and mania scales. The best correlations for the men given atropine at 104 µg/kg were with scores in the lie and mania scales. For the men given scopolamine at 11.8 µg/kg, the best correlations were with scores in the hypochondriasis and lie scales. If atropine and scopolamine are taken as representative of the group of tropic acid esters, the best indexes of performance in the Number Facility Test under the influence of such esters are the scores in the lie, hypochondriasis, and mania scales of the MMPI. After a similar analysis for five anticholinergic compounds, three other substances with atropine and scopolamine, Klapper et al. (162) decided that scores in the positive test-taking attitude, hypochondriasis, and mania scales of the MMPI were positively related to performance in the Number Facility Test.

Sidell et al. (164) reported that intravenous injection of the potent cholinesterase inhibitor, S-(diisopropylaminoethyl)-ethylmethylphosphonothioate, at 1.5-1.7 µg/kg into men 1.5 h after intramuscular injection of scopolamine hydrobromide at 24 µg/kg was effective in reversing the performance and cognitive decrements produced by the scopolamine. If the anticholinesterase was administered 30 min after scopolamine, the initial therapeutic benefit was less than when it had been given at 90 min. This anticholinesterase had a more durable effect than physostigmine, its antagonistic activity persisting as long as the agonistic activity of scopolamine, whereas that of physostigmine had been found by others to last for only 2.5-3 h.

Ketchum et al. (24,165,166) reported the results of intramuscular injection of atropine sulfate at 32-175 µg/kg, of intravenous injection of scopolamine hydrobromide at 5-24 µg/kg, of intramuscular injection of scopolamine hydrobromide at 5-24 µg/kg, of intramuscular injection of scopolamine methylbromide at 5-30 µg/kg, and of attempts to find antagonists of intoxication with these chemicals. The research involved 127 individual experiments. The ED_{50}s of atropine sulfate to initiate the following effects to recognizable extents were: poorly coordinated, 89 µg/kg; attention span shortened, 95 µg/kg; stumbling gait, 106 µg/kg; restlessness, 126 µg/kg; temporal confusion, 130 µg/kg; inability to obey simple orders, 135 µg/kg; and hallucination, 169 µg/kg. Although the ratios between the ED_{50}s of atropine and of the other anticholinergic compounds for the production of the different effects varied somewhat, intravenously injected scopolamine hydrobromide was about 8.7 times as active as atropine sulfate and, when injected intramuscularly, about 7.5 times as effective as atropine sulfate. Scopolamine methylbromide was about 4 times as active as atropine sulfate, except that no dose of the former substance produced as much as a 90% decrease in performance in the Number Facility Test, so comparative potencies had to be assigned on the basis of less than maximal actions by atropine and scopolamine. Therefore, scopolamine methylbromide was only about 53% as potent as the hydrobromide.

Intramuscular injections of scopolamine hydrobromide at 24 µg/kg and of atropine sulfate at 175 ug/kg induced approximately equal decrements in performance in the Number Facility Test, except that the maximal effect after scopolamine was attained in about half the time required to reach that after atropine. Intravenously injected scopolamine hydrobromide produced its maximal effect even more rapidly than that injected intramuscularly. Scopolamine methylbromide was slower in exerting its maximal tachycardial effect than scopolamine hydrobromide, but no information about the rapidity of its action on performance in the Number Facility Test was provided in any of the three reports (24,165,166). The increase in heart rate induced by scopolamine methylbromide was greater than those induced by the same amounts of scopolamine hydrobromide or atropine sulfate.

The decreases in performance in the Number Facility Test induced by atropine or scopolamine were antagonized rapidly by intramuscular injections of physostigmine at 15-60 µg/kg, but the antagonistic effect wore off after 3-4 h. Neostigmine had no immediate effect on performance in the Number Facility Test, but may have increased slightly the rate of spontaneous recovery 5-10 h after the intramuscular dose of atropine sulfate. With respect to the tachycardia induced by atropine, neostigmine may have exerted a prompter and more potent bradycardiac action than physostigmine. Physostigmine also antagonized the decrement in performance on the Number Facility Test induced by scopolamine. The organophosphorus anticholinesterase compounds, DFP and Sarin, had some antagonistic effect against the decrement in performance in the Number Facility Test induced by scopolamine, but these effects were less striking, although more persistent, than those of physostigmine. Tetrahydroaminoacridine, also an inhibitor of cholinesterases, had some antagonistic activity against the decrease in performance in the Number Facility Test due to scopolamine. Intramuscular injection of chlorpromazine after a small dose of scopolamine hydrobromide (8 µg/kg) increased the decrement in performance in the Number Facility Test due to the scopolamine. When 50 mg of chlorpromazine was injected intramuscularly after intramuscular scopolamine hydrobromide at 8 µg/kg, the resulting decrease in the score in the Number Facility Test approached that induced by scopolamine hydrobromide at 24 µg/kg alone, although chlorpromazine by itself induced only a slight decrease in performance. Perphenazine, another derivative of phenothiazine, had effects similar to those of chlorpromazine.

Sidell (167) reported that absorption of atropine from an intramuscular site of injection in man was prolonged when it was mixed with a 30% solution, but not when mixed with an 18% solution, of pralidoxime chloride. Because absorption of atropine was also delayed when it was mixed with an 8.5% solution of sodium chloride, Sidell suggested that the delayed absorption with 30% pralidoxime chloride may have been due to osmotic effects, rather than to any specific effect of the oxime. However, as pointed out by Sidell, although absorption of atropine from the mixture with 8.5% sodium chloride was delayed, eventually this mixture caused the same mean increase in heart rate in the six volunteer subjects as atropine alone at the same time after injection. In contrast, neither mixture of atropine with pralidoxime chloride accomplished this; the mixture of atropine with the 18% solution came closer than the mixture with the 30% solution to achieving the same increase in mean heart rate as atropine alone. Therefore, pralidoxime may have had a dose-related effect on the total absorption of atropine. Whether this is due to a simple osmotic effect on the absorption of atropine, with some of the alkaloid held at the site of injection by unabsorbed pralidoxime chloride in solution, or to some more specific effect of the oxime is not clear.

Sidell (168) studied the effectiveness of intravenous administration of physostigmine as an antagonist of the central (Number Facility Test) and peripheral (heart rate) actions of atropine and scopolamine. The report simply reiterated the effectiveness of physostigmine as an antagonist of the central actions of these two alkaloids and demonstrated that repetition of administration of physostigmine after disappearance of the effect of a first dose renewed the antagonism of persistent central actions of the alkaloids, but, like the first dose of physostigmine, had only a minor effect on the tachycardia due to the alkaloids.

SUMMARY

This review of the general literature portrays the tropic acid esters with which this report is concerned as compounds that are not particularly lethal in single or repeated doses. The members of the group that are tertiary amines may affect psychic activity in comparatively small doses, but there is fairly rapid adaptation to repeated doses. Removal of the compounds from the body, so far as it has been studied, seems to be comparatively rapid and to occur by a combination of urinary and fecal excretion and metabolic transformation. The transport of intact atropine through the tubular epithelium of the chicken is by the cation transport mechanism and is reduced by drugs and chemicals that inhibit that transport process: quinine, choline, cocaine, and cyanine dyes (146).

Symptoms and signs of the action of the tropic acid esters on human subjects include complaints of mouth dryness and decreased secretion of saliva; increase in heart rate, possibly preceded by a short period of decrease in heart rate; dryness and flushing of the skin, particularly in the "blush area"; difficult urination; ataxia; disorientation; hallucination; delirium; dilated pupils, possibly nonreactive to light; prostration; and coma.

The greatest hazard to life arises from suppression of the ability to secrete sweat, which can give rise to fatal hyperthermia if body temperature is not controlled artificially during hot weather or strenuous activity. Another source of hazard to life arises from the effect of the compounds other than scopolamine and its quaternary form in accelerating heart rate and facilitating intramural conduction and transmission of impulses. These actions may result in serious arrhythmias up to and including ventricular fibrillation. The quaternary amine forms of the tropic acid esters in which we are interested are more active in some respects than the tertiary amines, as far as peripheral actions are concerned. This is especially true with respect to actions on nicotinic effectors in ganglia and striated muscles. The quaternary amines penetrate into the central nervous system poorly, but, once there, affect muscarinic effectors in the same way as the tertiary amines.

The anticholinergic compounds that are esters of tropic acid are not known to have any important mutagenic, teratogenic, or carcinogenic actions. There has been little study of long-term toxicity of these compounds, however, so one cannot say categorically that they have no carcinogenic activity. The rapidity with which atropine is removed from the body, although it cannot be taken as proof of noncarcinogenicity, does militate against a high probability of such an action by this compound.

ESTERS OF BENZILIC ACID

The master file of the Board on Toxicology and Environmental Health Hazards lists four compounds that are esters of benzilic acid. One of these compounds seems to be an isomer of another--both are benzilic acid esters of tropine. Of the 12 reports that concern administration of compounds of this group to volunteers, either at Edgewood Arsenal or at contractors' facilities, 11 are related to the results of studies with quinuclidinyl benzilate and three with tropinyl benzilate (these three reports are concerned exclusively with the L isomer of the ester). Accordingly, the literature review in this section centers on these two compounds insofar as possible, and especially on quinuclidinyl benzilate.

The first compound made in this group of esters of benzilic acid seems to have been N-diethylaminoethyl benzilate (benactyzine), whose synthesis was reported in 1938 by

Horenstein and Pahlicke (169). The synthesis of tropinyl benzilate was described in 1952 by Hromatka _et al_. (170). In 1952, Sternbach and Kaiser (171) reported methods for producing several bicyclic alcohols, including 3-quinuclidinol. These alcohols were then used to produce esters with organic acids (172), including benzilic acid. The esters were examined for their ability to induce relaxation of isolated rabbit intestine that was made to contract by acetylcholine; the esters of 3-quinuclidinol were found to have greater spasmolytic activity than the corresponding esters of diethylaminoethanol and other bicyclic alcohols. The 3-quinuclidinyl ester of diphenyl acetic acid was prepared in its L and D forms. The levorotatory ester was found to have most of the activity, the dextrorotatory ester having only very slight activity. The toxicities of the isomers were stated to have been identical, but no data on toxicity were reported. A paper by Randall _et al_. (173) gave the intravenous LD_{50}s for the mouse of the L isomer as 29.5 mg/kg and of the D isomer as 27 mg/kg.

The synthesis of esters of 2-tropanol was described by Archer and Bell (174) in 1961. In 1971, Atkinson and McRitchie (175) reported methods for synthesizing all four of the isomers of 2-tropanol. The chemical literature seems not to reveal when the production of L-2-tropinyl benzilate actually occurred, but it was probably in 1971-1972.

Randall _et al_. (173) assessed the potencies of 3-quinuclidinyl benzilate hydrochloride and _N_-methyl-3-quinuclidinium benzilate bromide in several different activities. The LD_{50}s of 3-quinuclidinyl benzilate for the mouse were about 1.46 and 2.04 times those of the quaternized compound after intravenous and intraperitoneal injection, respectively. In producing relaxation of isolated intestinal smooth muscle stimulated by acetylcholine, 3-quinuclidinyl benzilate was somewhat more effective than atropine. The 3-quinuclidinyl benzilate had some activity in relaxing spasm of intestinal smooth muscle induced by histamine and was quite effective in antagonizing that induced by barium. It was also effective in preventing contraction by bronchial smooth muscle on exposure to aerosolized methacholine and in overcoming the bradycardia and hypotension induced by injection of acetylcholine or by electric stimulation of the vagal nerves. A dose of 2 mg/kg prevented the response of the cat nictitating membrane to electric stimulation of the cervical sympathetic nerve.

The chemist who made 3-quinuclidinyl benzilate took about 0.5 mg of the compound and experienced not only dryness of the mouth and mydriasis, but also a sensation of weakness in the knees. Despite having received several subcutaneous doses of neostigmine, he slept fitfully that night and spoke incoherently during waking periods. On the next morning, he was still unsteady on his feet, and his pupils were dilated. By the second morning after he had taken the compound, all symptoms except those due to persistent mydriasis had disappeared. He revealed that he had felt confused and that time had seemed to pass very slowly. Two other employees involved in work with 3-quinuclidinyl benzilate had similar illnesses, with apparent hallucination in one patient (176).

In addition to those three occupational exposures, two of six patients given 0.1 mg of 3-quinuclidinyl benzilate three times a day as a treatment for parkinsonism had nocturnal confusion (176).

STUDIES IN ANIMALS

In an attempt to explain these confusional states, Schallek and Smith (177) studied the effects of five spasmolytic substances, including 3-quinuclidinyl benzilate and its quaternized form, on the electroencephalogram, the electrocardiogram, and the blood pressure of the dog. Intravenous injection of 3-quinuclidinyl benzilate at 0.01 mg/kg decreased the dominant frequency of the EEG of two of five dogs; a dose of 0.1 mg/kg enlarged to three the number of dogs in which the dominant frequency of the EEG was decreased. Larger doses, up to 10 mg/kg, did not further enlarge the proportion of dogs with a decreased dominant frequency. The dose of 0.1 mg/kg induced bradycardia in one of five dogs. A dose of 10 mg/kg induced bradycardia in all five dogs and resulted in death due to cardiac arrest in two of five.

The only dose of quaternized 3-quinuclidinyl benzilate that induced any alteration in the EEG was 10 mg/kg, which resulted in a decrease in the dominant frequency in the EEG of one of five dogs. This dose also induced bradycardia in three of five animals and hypotension in all five. No dogs given this dose of *N*-methyl-3-quinuclidinyl benzilate bromide died. Intravenous doses of 0.1 and 1.0 mg/kg caused tachycardia in one of five and two of five dogs, respectively.

Before proceeding with a discussion of the effects of 3-quinuclidinyl benzilate, it may be worth while to look at the available information on the lethality of single doses of the benzilic acid esters with which we are concerned. Table I-2 is based on information supplied by Hoffman-LaRoche, Inc., the Sterling-Winthrop Research Institute, and a number of investigators (36,173,176,178-189).

The benzilic acid esters of both diethylaminoethanol (179, 180,185) and tropine (182,183) were found to be capable of overcoming spasms of smooth muscle induced by acetylcholine and of inducing relaxation of smooth muscle, such as the circular muscle of the iris. Both compounds had some local anesthetic activity, both produced changes in the EEGs of experimental animals similar to those induced by atropine, and both had slight antihistaminic activity. Lisunkin (187) reported that tropinyl benzilate increased the effectiveness of other antispasmodic drugs, such as tropinyl diphenylacetate (tropacine), in antagonizing the tremor induced by subcutaneous nicotine.

Larsen (181) fed rats diets containing either 0.01% or 0.05% of the benzilic acid ester of diethylaminoethanol from 28 d of age until spontaneous death. Another group of rats was fed the basal diet. The rats fed benzilic acid ester gained weight slightly faster than those fed the normal diet, but died younger than the controls (means of 24 and 26 mo, compared with one of over 29.5 mo). No striking pathemas in the animals due to the benzilic acid ester were reported.

3-QUINUCLIDINYL BENZILATE (BZ, EA 2277)

McNamara (184) reported that rats placed into atmospheres containing 3-quinuclidinyl benzilate dispersed as a dust, as a thermally generated smoke, or as an aerosol of a 1% solution of the benzilic acid ester in methylene dichloride for concentration-time (Ct) products up to 29,508 mg.min/m^3 experienced no permanent effects from the exposures, except occasional deaths randomly distributed with respect to intensity of exposure. One of four guinea pigs exposed to a Ct product of 10,123 mg.min/m^3 and two of four guinea pigs exposed to a Ct product of 14,748 mg.min/m^3 died. Mice exposed to an aerosolized 1% solution of the benzilic acid ester in methylene dichloride at Ct products up to 14,137 mg.min/m^3 did not experience any clearly dose-related mortality, but 69 of 70 mice exposed to thermally generated smoke of 3-quinuclidinyl benzilate at Ct products of 8,213-9,331 mg.min/m^3 died.

McNamara (184) reported that daily subcutaneous doses of 3-quinuclidinyl benzilate at up to 150 µg/kg on 5 days of each week for 3 wk induced no evident signs of toxicity in mice and guinea pigs. Dogs given daily intravenous doses of 100 µg/kg for 14 consecutive days had the same LD_{50} as dogs that had not been pretreated, but the time between injection of the daily dose and the appearance of ataxia increased from 4 min to 14 min during the period of pretreatment. In dogs trained in a conditioned-escape routine and then given graded intravenous doses of 3-quinuclidinyl benzilate at up to 12.5 µg/kg, there was no effect on performance. A dose of 25 µg/kg resulted in failure to escape by four of four dogs.

The same report (184) summarized data on the effect of intraperitoneally injected 3-quinuclidinyl benzilate on spontaneous motor activity of the rat. When the data were plotted and the curve extrapolated to zero change in spontaneous activity, the maximal dose that could be given without altering spontaneous activity seemed to be about 0.12 mg/kg. No rats were given daily injections of saline, however, so a decision on what portion of the observed changes was attributable to the compound, rather than to the procedure, is not possible.

Studies of the absorption of 3-quinuclidinyl benzilate from solutions applied in a mixture of alcohol and cresol to the clipped skin of five species of animals, summarized in the same report (184), found that doses of up to 500 µg/kg produced ataxia in the cat after about 30 min and in the dog after about 55 min. No ataxia was produced in the goat or the monkey (probably rhesus) by the highest dose; the highest dose applied to the skin of the rabbit was 100 µg/kg. Doses of 50 µg/kg applied to the skin of the rabbit, of 100 ug/kg applied to the skin of the cat, of 250 µg/kg applied to the skin of the monkey, and of 500 µg/kg applied to the skin of the dog and the goat were the lowest doses found to have effects on the eyes. In all species except the monkey, recovery from the ocular effects was stated to occur overnight. In the monkey, the ocular effects apparently persisted for 2 d or more.

In the dog, an intravenous dose of 10 µg/kg produced a mean increase in four dogs of about 143% in heart rate, which decreased slowly during an observation period of up to 6 h, but was still evident at the end of that period. By 24 h after the dose, the heart rate was back to normal. A dose of 12.5 µg/kg, but not one of half that amount, decreased by 20% the mean time during which dogs would run on a treadmill. None of the experiments summarized in this report (184) seems to have involved any persistent effect of exposure to 3-quinuclidinyl benzilate in surviving animals. Neither did the report of Wal (186) mention any consistent toxic effects of this compound.

Ketchum *et al*. (189) reported that rats and dogs with whole-body exposures and rabbits and monkeys with head-only exposures to clouds of 3-quinuclidinyl benzilate dispersed in the field at wind speeds of 8-11 mph developed such effects as ataxia, dyspnea, mydriasis, cycloplegia, tachycardia, sedation, hyperactivity, and nasal stuffiness. The most persistent of these effects was cycloplegia, which persisted in three of five monkeys for more than 7 d. Most other effects disappeared within 48 h after exposures.

No study of long-term administration of 3-quinuclidinyl benzilate to experimental animals has been found. The closest approaches to such a study are 1-yr studies of the results of feeding the methyl bromide of 3-quinuclidinyl benzilate to rats in the diet (190) and of gavaging dogs 5 d/wk with the same quaternary amine salt of 3-quinuclidinyl benzilate (191). Rats were fed diets containing the quaternary amine salt at concentrations calculated to yield daily doses of zero, 5, 25, and 50 mg/kg. Dogs were gavaged with the quaternary salt at zero, 1, 5, and 25 mg/kg. In both experiments, no evidence of dose-related toxicity was seen. The livers of the male dogs that had received daily doses of 5 and 25 mg/kg seemed to be larger than those of the controls (3.0% and 3.2% of body weight, respectively, versus 2.7% for the controls), but were normal in structure on necropsy and in function (glutamic-pyruvic transaminase and alkaline phosphatase activities in serum and Bromsulphalein retention within normal limits). Although the compound used in these two studies was not 3-quinuclidinyl benzilate and probably was absorbed from the intestinal tract less rapidly and less completely than that compound, it contained the same combinations and arrangements of atoms. Therefore, the negative findings with respect to organ and tissue damage in the two studies may indicate that similar experiments with 3-quinuclidinyl benzilate would result in no chemically induced pathemas.

The great avidity of mitochondria from rat brain for 3-quinuclidinyl benzilate has been mentioned previously (56). Larsson *et al*. (192) recorded the infrared spectra of seven esters of benzilic acid, of eight esters of 3-quinuclidinol, and of atropine and scopolamine and attempted to correlate the relative strengths of the intramolecular hydrogen bond with the threshold doses of the various compounds in producing psychotomimetic effects in dogs. The data of Albanus (42) on the psychotomimetic activities of anticholinergic compounds were used for the comparison. Of the esters of benzilic acid, the most active was 3-quinuclidinyl benzilate. According to Albanus's data, it was 50 times as active as tropinyl benzilate. Four other esters of 3-quinuclidinol were as active as 3-quinuclidinyl benzilate, but none was more active. Larsson et al. were not able to detect any correlation between the strength of the hydrogen bond, as derived from their spectra, and the biologic activities of the compounds estimated by Albanus.

Dogs given subcutaneous doses of tropinyl benzilate at 0.50 mg/kg became ataxic after a mean elapsed time of 16 min and obstinately progressive after a mean of 19 min; ataxia disappeared after a mean elapsed time greater than 283 min, whereas obstinate progression lasted only a mean of 244 min. Dogs given doses of 3-quinuclidinyl benzilate at 50 µg/kg became ataxic after a mean of 36 min and became obstinately progressive after a mean of 42 min. These two effects disappeared at mean times of greater than 313 and greater than 305 min, respectively. Tropinyl benzilate in a dose 10 times that of 3-quinuclidinyl benzilate produced toxic effects somewhat more rapidly than the latter, but these effects were less durable than those of the smaller dose of 3-quinuclidinyl benzilate.

Lavallee (50) reported that doses of 3-quinuclidinyl benzilate at 10 and 18 µg/kg had no progressive effects on performance of a multiple-stimulus conditioned-avoidance response by dogs, but doses of 24 and 32 µg/kg decreased the number of correct responses progressively. Monkeys given doses of 32 and 56 µg/kg may have made a few more errors in a visual-discrimination avoidance task than monkeys given no compound, but the difference was not statistically significant in the absence of control data.

Cats given intravenously injections of 3-quinuclidinyl benzilate labeled with ^3H in the 3-position of quinuclidinol were used to determine the distribution of the label in the brain and brain stem (193). Taking 304 counts/min.mg in the nervous tissue as the halfway point between the highest and the lowest concentrations measured, the areas of the brain that retained the greatest concentrations of the label were motor cortex, sensory cortex, caudate nucleus, lateral geniculate, and medial geniculate (167, 140, 121, 107, and 101 counts/min.mg, respectively). The areas that retained smaller concentrations, in order of decreasing concentration of the label, were thalamus, hippocampus, hypothalamus, medulla oblongata, colliculi, cerebellar cortex, pyramids of the medulla, cerebral white matter, and cerebellar white matter.

Both cholinomimetic and anticholinergic compounds were found (193) to reduce the retention of the label in some areas of the brain, the most strongly affected areas varying with the compound. The cholinomimetic compounds used were tetrahydroaminoacridine (THA) and S-diethylaminoethyldiethylphosphorothioate (TETRAM), both inhibitors of cholinesterases. The anticholinergic compounds used were N-methyl-4-piperidinyl- (phenylcyclopentyl)-glycolate (EA 3443) and 302,028 (not identified except as an analogue of 3-quinuclidinyl benzilate). The last compound had a much more general action than any of the others used, displacing the label of 3-quinuclidinyl benzilate from most of the sampled areas of brain and brain stem. In the cases of both EA 3443 and 302,028, displacement of the label was related probably to the substitution of one intoxicant by another, so that neither of these two anticholinergic substances would be practical antagonists of intoxication induced by 3-quinuclidinyl benzilate. Antagonism might reasonably be expected from THA, which partly displaced the label from motor cortex, lateral geniculate, medulla, and cerebellar cortex; antidotal activity by TETRAM--which displaced the label from lateral geniculate, thalamus, and hippocampus--is less likely. No attempts to assess antidotal effectiveness were made in this study.

Zvirblis and Kondritzer (56) found that a mitochondrial fraction prepared from rat brain adsorbed 3-4 times as much 3-quinuclidinyl benzilate as atropine in the physiologic range of pH. The optimal pH for adsorption of 3-quinuclidinyl benzilate was about 8.0, whereas that for atropine was about 9.7; at these pHs, the adsorption of the two anticholinergic compounds was about the same. THA decreased the adsorption of the benzilate by mitochondria, but a ratio of 100:1 (THA:3-quinuclidinyl benzilate) was required to reduce the adsorption by 50%. Both Ca^{2+} and PO_4^{2-} decreased the mitochondrial adsorption of 3-quinuclidinyl benzilate, the effect of Ca^{2+} not being particularly large and requiring an estimated ratio of 30,000:1 (Ca^{2+}:3-quinuclidinyl benzilate) to decrease adsorption of the benzilate by 50%. With phosphate, a ratio of about 21.5:1 (PO_4^{2-}:3-quinuclidinyl benzilate) would be expected to decrease adsorption of the benzilate by 50%.

Yamamura et al. (194) studied the adsorption of ^3H on particles in homogenates of various regions of monkey brain after incubation with $[^3H]$3-quinuclidinyl benzilate in solution in phosphate buffer at a pH of 7.4. The regions that were the most avid adsorbers of the benzilate, in order of decreasing uptake, were putamen, caudate nucleus, occipital cortex, cingulate gyrus, postcentral gyrus, hippocampus, amygdala, precentral gyrus, pyriform cortex, frontal cortex, superior colliculi, thalamus, and inferior colliculi. This distribution of adsorptive sites among various parts of monkey brain differs from that for uptake of ^3H from intravenously injected $[^3H]$3-quinuclidinyl benzilate by cat brain (193). The two studies are in agreement that the caudate nucleus is one of the most important areas of localization of the label in the form of $[^3H]$3-quinuclidinyl benzilate. Yamamura et al. found that uptake of the label by various areas of brain had a tendency to be correlated with choline uptake and with choline acetylase activity.

Yamamura and Snyder (195) found that interruption of the septal hippocampal tract, and elimination thereby of cholinergic afferents to the hippocampus, reduced by about 70% the activity of choline acetylase in homogenates of the hippocampus, but did not alter the binding of $[^3H]$3-quinuclidinyl benzilate by the particles of such homogenates. They proposed, as a possible explanation, that presynaptic muscarinic sites that are innervated by the septal hippocampal nerve fibers do not adsorb the labeled benzilate, but that postsynaptic muscarinic sites just across the synaptic gap from the presynaptic ones do adsorb it. Another possibility mentioned is that there may be no presynaptic muscarinic sites in the hippocampus.

The ATPase in microsomes prepared from whole rat brain, from rat cerebral cortex, and from cerebral cortex of rats that had received 3-quinuclidinyl benzilate intraperitoneally at 1 mg/kg an hour before they were killed was found (196) to be enzymatically competent in all cases and not to be inhibited significantly by the addition of 3-quinuclidinyl benzilate in vitro. Furthermore, slices of guinea pig brain cortex incubated in vitro with 10^{-4} M 3-quinuclidinyl benzilate had the same patterns of loss of K^+ and uptake of Na^+ as slices incubated without the benzilate. Inasmuch as ATPase is related intimately to the active transport of the monovalent cation in nervous tissue, the experiment with the brain slices gave an additional indication that ATPase activity is not altered by 3-quinuclidinyl benzilate.

Jovic and Zupanc (197) measured the effects of 3-quinuclidinyl benzilate on oxygen consumption by rats and oxygen uptake by slices of rat cerebral cortex and medulla oblongata. Subcutaneous injections of the benzilate at 4-15 mg/kg decreased oxygen consumption by a mean of 12.1%; there was no clear dose-effect relationship. Rats whose oxygen consumption had been increased by a mean of 33.5% by a small dose of soman (25 ug injected subcutaneously) had the increase limited to about 2% by a subcutaneous dose of 3-quinuclidinyl benzilate at 15 mg/kg.

Slices of cerebral cortex and medulla oblongata stimulated by KCl at a final concentration of 100 mM increased their oxygen uptake by respective means of 59.2% and 41.7% (197); inclusion of 5×10^{-4} M 3-quinuclidinyl benzilate in the medium bathing the slices reduced the stimulated oxygen uptake to 16.3% for cerebral cortex and to 3.6% for medulla oblongata. When rats were given 3-quinuclidinyl benzilate subcutaneously at 5-20 mg/kg and were killed 1 h later for preparation of slices of cortex and medulla oblongata, there were dose-related decreases in oxygen uptake by both tissues stimulated by inclusion of KCl in the fluid surrounding the slices.

Frances and Jacob (81) reported that intraperitoneal injections of 3-quinuclidinyl benzilate into mice at 0.3-3.0 mg/kg decreased the concentration of acetylcholine that could be measured in the brain. There was a linear relation between the decrease in acetylcholine concentration and the logarithm of the dose of benzilate. The line for this relationship with 3-quinuclidinyl benzilate was close to and nearly parallel with that with scopolamine.

Aquilonius *et al.* (77) found that intravenous 3-quinuclidinyl benzilate at 50 µg/kg produced about the same loss of acetylcholine from the rat cerebral cortex as atropine at 750 µg/kg. Intravenous 3-quinuclidinyl benzilate at 0.15, 0.2, and 0.5 mg/kg produced greater initial increases in rat locomotor activity than atropine at 1.3, 2, and 10 mg/kg. The immediate responses to the benzilate were not so well maintained as those to atropine. As the benzilate dose was increased from 0.1 to 0.2 mg/kg, locomotor activity increased rapidly to an almost maximal value that was about 9.3 times the control value.

The concentration of acetylcholine in rat hippocampal tissue was increased slightly (10.3%) by intraperitoneal 3-quinuclidinyl benzilate at 0.1 mg/kg (79); after a dose of 5 mg/kg, the acetylcholine concentration was reduced by 44.5%, compared with the mean concentration found in control rats. When septal lesions were created, the mean concentration of acetylcholine in the hippocampus increased by about 47.4%. As in intact rats, administration of 3-quinuclidinyl benzilate at 0.1 mg/kg increased the mean concentration of acetylcholine in the hippocampus, in this case by 5.3%. Unlike the result in intact rats, administration of 5 mg/kg resulted in a further increase in the mean concentration of acetylcholine in the hippocampus, to 16.6% above that in control rats. This last result was the converse of that found with atropine, but was similar to that found with scopolamine.

Meyerhöffer (198) found that L-3-quinuclidinyl benzilate was about 20 times as active as the Disomer in dogs in producing ataxia, tachycardia, and obstinate progression and in reducing salivation.

Kabes et al. (199) reported that rats given doses of 0.8, 2.0, and 5.0 mg/kg of 3-quinuclidinyl benzilate (by an unidentified route) had dose-related increases in spontaneous motor activity. Physostigmine (0.2 mg/kg), amobarbital (20 mg/kg), and chlorpromazine (2 mg/kg), all given by unidentified routes before the benzilate, held the spontaneous motor activity even below the control values.

Kabes (200) examined the effects of 3-quinuclidinyl benzilate on the abilities of rats to learn a conditioned-escape task and, once trained, on performance of the task. Doses of 0.5, 1, and 2 mg/kg of the benzilate hastened acquisition of the conditioned response in a dose-related manner, but a dose of 1 mg/kg had different effects on performance of the task in relation to the proficiency of the animal in its performance before the benzilate was administered. Rats that were poor performers (more than 20% errors in control sessions) and some that were good performers (less than 20% errors in control sessions) had their performance improved by the benzilate, whereas other good performers had their performances worsened.

Kabes (201) examined the effects of 3-quinuclidinyl benzilate on beagles. There were dose-related responses to doses of 10 and 50 µg/kg in autonomic functions (heart rate, pupil diameter, flow of saliva, etc.), motor functions (motor coordination, tremor, ataxia, etc.), and central nervous system functions (alertness, orientation, reaction to external stimuli, etc.). Physostigmine (1 mg/kg) aborted all these types of action. Routes of administration were not stated; physostigmine was said to have been injected, but the site was not stated.

Fusek et al. (202) used isolated rat jejunum and atria to examine the effects of 3-quinuclidinyl benzilate on tissue response to cholinergic agonists. The benzilate alone had no effect on isolated jejunum, but, when applied before a stimulant concentration of furtrethonium (10^{-7}-10^{-4} M), it shifted the dose-response curve for furtrethonium to the right, i.e., a higher concentration of furtrethonium was required to produce a given response in the presence of 3-quinuclidinyl benzilate than in its absence.

The application of 3-quinuclidinyl benzilate at a range of concentrations (3-30 x 10^{-9} M) to isolated atria had no effect on the contractions of either spontaneously beating or electrically driven atria (202). When the atria were subjected to the influences of both furtrethonium and 3-quinuclidinyl benzilate, the dose-effect curve for the former was shifted to the right. This was similar to the result with isolated jejunum, the molar ratio of furtrethonium to 3-quinuclidinyl benzilate being about 3.3 x 10^6:1.

Herink et al. (82) injected 3-quinuclidinyl benzilate at 1 mg/kg into the peritoneal cavities of normal rats and rats with electrolytic septal lesions. In normal rats, there was no significant effect on aggressiveness, but defecation was decreased. In the rats with septal lesions, both aggressiveness and defecation were reduced by 3-quinuclidinyl benzilate (the latter being affected to the greater extent).

Poel (203) trained female rats to run in a circular runway for a reward of a drop of chocolate paste. After completion of training, the rats were given intraperitoneal injections of 3-quinuclidinyl benzilate at 0.1, 0.3, 1, 3, or 10 mg/kg and were placed in the runway 20 min later. The benzilate induced a marked increase in the time required for the rat to leave one reward area and begin to seek a second reward (latency); the increase in latency was dose-related. There was a dose-related increase in the time required for the rat to traverse the circular runway (running time), but the slope of the regression line for the relationship of running time to the dose of 3-quinuclidinyl benzilate was much smaller than that for the relationship of latency to dose. These results were interpreted as indicating that the benzilate intensified a conflict between a desire to remain in the vicinity of a prior reward and a desire to obtain a later reward, coupled with some interference with motor function similar to that found by Meyerhöffer (198) and Kabes et al. (199,200).

Bell and Gershon (86) found that intravenous injection of 3-quinuclidinyl benzilate at 50 ug/kg into dogs induced intoxication that persisted for more than 5 h. Intravenous injection of 1,2,3,4-tetrahydro-9-aminoacridine (Tacrine or THA) at 1 mg/kg within 30-50 min after administration of the benzilate induced recovery in only two of nine dogs, but similar treatment 2-3 h after the benzilate had been injected was successfully antidotal in seven of seven dogs. Yohimbine, which has cholinomimetic and sympatholytic actions (204), injected intravenously at 0.5 mg/kg within 30-50 min after the benzilate had antagonistic activity in four of nine dogs. When yohimbine was not administered until 2-3 h after the benzilate, it antagonized the intoxication in only two of seven dogs. Piperoxan, also an adrenergic blocking agent, was mentioned as being capable of antagonizing intoxication with 3-quinuclidinyl benzilate, but no details of study of its action were given.

Fusek et al. (205) reported that Tacrine inhibited both acetylcholinesterase and butyrylcholinesterase in dog lungs and heart and slightly inhibited acetylcholinesterase in the brain, especially in basal ganglia. Most of the change in the acetylcholinesterase activity of the brain was found to be in the microsomal fraction. Butyrylcholinesterase in the bodies of ganglion cells was said to be completely inhibited in all regions of the brain. These authors found that an intramuscular dose of tacrine at 10 mg/kg completely abolished within 2 h the signs of intoxication induced by an intramuscular injection of 3-quinuclidinyl benzilate at 50 µg/kg.

Sram et al. (206) reported the results of cytogenetic analyses of spermatozoa from mice that had been given intraperitoneal 3-quinuclidinyl benzilate in single injections at 40 mg/kg or 15 daily doses at 10 mg/kg, of bone marrow cells from mice that had received 15 daily doses at 2 or 10 mg/kg, and of human lymphocytes that had been exposed in vitro at 10^{-4}-10^{-3} M. They looked also for reverse mutations in yeast cells exposed in vitro at 5×10^{-4} M and 5×10^{-2} M, for host-mediated transformations in yeast cells injected into the peritoneal cavities of mice that were then given 50 or 100 mg/kg subcutaneously, and for dominant-lethal effects in mice after single intraperitoneal injections at 10 or 40 mg/kg or repeated injections--15 at 2 mg/kg, 15 at 10 mg/kg, or 5 at 20 mg/kg. The yeast cells exposed to 3-quinuclidinyl benzilate in vitro gave evidence of weak mutagenic activity. The only other positive results were in bone marrow cells of mice given doses of 10 mg/kg or more. Sram et al. (206) concluded that, because the frequency of chromosomal breaks was not dose-related above the threshold dose for the occurrence of breaks, the breaks were probably due to systemic toxic activity by the benzilate, rather than to any specific mutagenic effect, and that the compound has no serious genetic risk for man.

In another paper, Sram (207) examined the production of chromosomal abnormalities in bone marrow from male Chinese hamsters given single intraperitoneal injections of 3-quinuclidinyl benzilate at 0.1, 1, 10, 20, 40, or 60 mg/kg, dissolved in dimethyl sulfoxide. Control hamsters received dimethyl sulfoxide alone. Doses of 1 mg/kg or more induced more chromosomal gaps and breaks per cell than the solvent. Doses of 1-40 mg/kg yielded apparently dose-related increases in the incidence of chromosomal abnormalities: 0.012 per cell with 1 mg/kg, 0.064 per cell with 10 mg/kg, 0.224 per cell with 20 mg/kg, and 0.292 per cell with 40 mg/kg. The highest dose, 60 mg/kg, produced only 0.076 per cell. Sram considered, on the basis of the count with the highest dose, that the incidence of chromosomal gaps and breaks was not dose-related, but that is questionable for the doses between 1 and 40 mg/kg. However, as Sram pointed out, chromosomal gaps and breaks seem to be evidence of toxic damage to the individual, rather than of heritable change, so they probably do not indicate a serious genetic risk for man.

Hughes et al. (208) gave 10 beagles intravenous 3-quinuclidinyl benzilate at 3 mg/kg twice a week for 3 wk. Five control dogs were given 0.1 N HCl, corresponding in volume and pH to the solution of benzilate at 0.1 ml/kg. Some animals from each group were killed 72 h after the last dose of benzilate, and the others were killed 60 d after the last dose. No lesions attributable to benzilate were found in the testes of the dogs that had been given that compound.

DIETHYLAMINOETHYL BENZILATE

The only other member of the group of esters of benzilic acid on whose effects there is a considerable body of information is diethylaminoethyl benzilate. One review of its pharmacology has been mentioned previously (181); it concerned the general pharmacology of the drug as a peripherally effective muscarinic blocking agent. In addition to having cholinergic spasmolytic activity, diethylaminoethyl benzilate also has mydriatic effectiveness, is able to antagonize barium-induced contraction of intestinal smooth muscle, has local anesthetic properties, and has an antiarrhythmic action on the heart similar to that of quinidine. In 1955, a series of papers established that diethylaminoethyl benzilate affects the functioning of the nervous system in the cat (209), the rat (210), and man (211).

Berger et al. (212) studied the toxicity of single doses of diethylaminoethyl benzilate in mice and monkeys and its general actions on several functional systems. In the monkey, an intravenous dose of 1 mg/kg resulted in mydriasis and some decrease in voluntary movement. A dose of 2 mg/kg induced ataxia with occasional convulsive jerks. A dose of 6 mg/kg induced intermittent clonic convulsions in one monkey; the convulsive state lasted for about 10 min and was followed by a depressed state lasting for over an hour. Pupillary dilatation, the effect of longest duration, sometimes persisted for as long as 20 h.

Diethylaminoethyl benzilate injected intraperitoneally into mice with hexolbarbital at 100 mg/kg (212) prolonged anesthesia due to the hexolbarbital: benzilate at 10 mg/kg increased the duration of anesthesia by 35.9 min (124%) and at 20 mg/kg increased it by 68.3 min (236%). When diethylaminoethyl benzilate was administered to mice by mouth at 24 mg/kg and the mice were then subjected to what was usually a nonlethal convulsigenic electric shock, 50% of the mice died. The only antimuscarinic effect detectable in these mice was mydriasis. This dose of diethylaminoethyl benzilate did not prolong the latency to onset of convulsion after delivery of the electric shock; a larger dose, 37 mg/kg, did prolong the latency significantly.

When different agonists (acetylcholine, 5-hydroxytryptamine, and histamine) were used (212) to stimulate the smooth muscle in isolated rat colon, diethylaminoethyl benzilate had some ability to antagonize the stimulant action of all three agonists. Its ability to block the action of acetylcholine was about 20 and 100 times its activity against the stimulant actions of 5-hydroxytryptamine and histamine, respectively. Diethylaminoethyl benzilate did not increase the urinary excretion by the rat of 5-hydroxyindoleacetic acid, but did magnify the pressor action of epinephrine and reduced its LD_{50}. The compound appears, therefore, not to affect the metabolism of serotonin, but to alter in some way the relation between epinephrine and its receptors.

Diethylaminoethyl benzilate had no significant effect on the knee-jerk or flexor reflex in the cat (212); when injected into curarized cats, it altered the predominant frequency of the EEG from 40 per second to 8-15 per second and induced enlarged discharges. The changes in the potentials recorded from the brain were seen in records from both cortical and subcortical regions. Electric arousal consequent to peripheral or thalamic stimulation of cerebral afferent fibers was blocked by diethylaminoethyl benzilate, but recruitment of cortical neurons by stimulation of the thalamus was not affected.

Giarman and Pepeu (75) reported in 1962 that diethylaminoethyl benzilate differed from atropine in that a dose equimolar with atropine at 50 mg/kg (which reduced the concentration of acetylcholine in rat brain by 33%) caused no significant change in the brain's concentration of acetylcholine. Both atropine and diethylaminoethyl benzilate were said to induce mild excitation, however. In contradiction of this report, Frances and Jacob (81) published a graph indicating that intraperitoneal atropine at 1 to 7 or 8 mg/kg had a greater effect in lowering the brain acetylcholine concentration in the mouse than doses of diethylaminoethyl benzilate in the same range, but that the benzilate at 25-30 mg/kg induced a greater lowering of the acetylcholine concentration than atropine.

McColl and Rice (213) found that intraperitoneal injection of mice with diethylaminoethyl benzilate at 10 mg/kg 15 min before similar administration of tremorine increased the ED_{50} of tremorine from 5.8 to 38.0 mg/kg. Such an effect can be explained as being due to depletion by the benzilate of the store of acetylcholine in the brain cells.

In 1964, Jacobsen published a review of the literature through 1962 on the actions of diethylaminoethyl benzilate (214). It reported that oral doses of this benzilate of up to 80 mg had been tolerated by man, but that daily doses of about 90 mg had given rise to mild arrhythmias and cyanosis. The compound had been found to enter the rat brain in unaltered form. When ^{14}C-labeled diethylaminoethyl benzilate had been administered to rats, 85% of the label had been recovered in the urine within 24 h. Nothing was known at that time about the nature of the metabolites. The compound had been found to inhibit oxidative phosphorylations in brain cells in vitro, and, at high concentrations (10^{-3} M), to inhibit monoamine oxidase. In man, doses of 40-200 mg of this benzilate had been found to reduce excretion of 5-hydroxyindoleacetic acid to zero. Thus, the compound interferes in some way with the utilization or metabolism of serotonin, and presumably with the function of serotonergic synapses. In addition to having peripheral antimuscarinic activity, graded by various investigators as between 0.01 and 0.33 that of atropine, diethylaminoethyl benzilate had been found to be particularly potent in blocking transmission through the ascending reticular system; it had also been found to affect the descending reticular system, but not to be useful in treating parkinsonism, because of its other central effects. Diethylaminoethyl benzilate inhibits the metabolism of barbiturates and potentiates their central depressant activity. In addition to inducing hyperactivity in mice and rats and reducing the reactions to stress in rats and cats, diethylaminoethyl benzilate had been found not to affect sham rage, to inhibit fighting between rats exposed to electric shocks, and to decrease the rate of learning of complicated tasks (tasks requiring discrimination between stimuli and the making of different responses to different stimuli) in mice, rats, and cats.

Berger et al. (80) reported that diethylaminoethyl benzilate was particularly effective in increasing the duration of after-discharges by the cat hippocampus after a weak, brief electric shock, being 40-100 times as active as atropine in this regard. As to its antispasmodic action in vitro and its ability to block salivation induced by intracranial injection of 80 ug of acetylcholine chloride into a mouse, diethylaminoethyl benzilate was 4% and 77%, respectively, as potent as atropine.

Shitov (215) found that benactyzine and two other anticholinergic compounds increased the action of magnesium sulfate on the EEG of the rabbit in proportion to the activity of the compounds in activating cholinesterase.

Banshchikov and Stoliarov (216) reviewed a group of substances that had been found to be capable of evoking mental disturbances. All the substances have marked anticholinergic activity, and diethylaminoethyl benzilate was among them. The others are Biel's compounds, JB-318, JB-329 (Ditran), and JB-336.

The authors (216) stated that usual medicinal doses of diethylaminoethyl benzilate not infrequently produce mental changes--retardation or stoppage of the current of thought, a feeling of emptiness, forgetfulness, shortened attention span, sensations of heaviness and altered shape of the limbs, apathy, sluggishness, impression of isolation from the environment, hostility, apprehension, horror, anxiety, and hypochondriasis. A relatively brief period of these effects might be followed by a period of euphoria if more than 5 mg of the benzilate had been taken.

The first report of a psychotomimetic episode due to diethylaminoethyl benzilate in a human being was credited to Vojtechovsky (217) by Banshchikov and Stoliarov (216). This investigator had published in 1958 a case report on a female physician who had accidentally taken about 1.4 g of the benzilate. The woman experienced visual hallucinations and brief delirium. Intentional administrations of diethylaminoethyl benzilate to human subjects had shown (218) that the psychotomimetic state lasted for 4-12 h and was accompanied by a decrease in urinary excretion of 5-hydroxyindoleacetic acid. These authors stated that diethylaminoethyl benzilate had been found to inhibit monoamine oxidase only slightly less strongly than iproniazid. As the psychotomimetic state waned, the urinary excretion of 5-hydroxyindoleacetic acid increased to such an extent that the 24-h excretion of this degradation product of serotonin was within normal limits. It had been found in the same experiment that the urinary excretion of 17-ketosteroids was markedly reduced during the period of psychosis and that the activity rebounded at about the same time as the urinary excretion of 5-hydroxyindoleacetic acid. In the case of the 17-ketosteroids, however, the rebound increased the total daily urinary excretion of 17-ketosteroids by nearly 50%.

Edelson et al. (219) used [^{3}H]diethylaminoethyl benzilate to study the absorption, distribution, and metabolic fate of this benzilate in the rat. The substance used was randomly labeled. After intraperitoneal injection of benzilate at about 82.5 mg/kg, ^{3}H was detectable in the blood at a fairly high concentration about 12 min after injection; the blood concentration was highest about 36 min after injection. At 5 h after injection, the ^{3}H concentration was about 28% of its peak. The authors estimated the half-time of the label in the blood to be about 80 min. If one calculates (from data in the paper) the time required for removal of half the peak ^{3}H concentration from the blood, that time is found to be about 114 min after the injection. The authors' value of 80 min for the half-time would require the concentration of label in the blood 5 h after injection to be about 6.7% of the peak concentration, whereas it was actually about 28% of the peak. The estimate of 114 min is believed to be a better value for the half-time.

Within 24 h after injection, 44.8% of the label was recovered in the urine and 41.6% in the feces and the gastrointestinal tract and its contents. The carcass contained 14.2% of the label, of which the greatest amount was in the liver. The expired air contained about 0.3% of the ^{3}H, in the form of water. The urine contained the unchanged compound, ethylaminoethyl benzilate, and benzilic acid. Apparently, a considerable portion of the diethylaminoethyl benzilate had been hydrolyzed to release benzilic acid, which accounted for 54.4% of the label in the urine. A smaller portion of the original compound had been monodealkylated to ethylaminoethyl benzilate, which accounted for 13.8% of the label in the urine. This dealkylation may have contributed the small amount of the label found in the water of the expired air. The fate of the diethylaminoethanol that must have been released by hydrolysis of the original compound remains unknown. A study of the hydrolysis in vitro of the original benzilate at a pH of 7.4 during incubation at 37°C revealed that approximately 50% of the compound underwent spontaneous hydrolysis within 5 h.

Sram (207) gave Chinese hamsters diethylaminoethyl benzilate intraperitoneally at 0.1, 1, and 10 mg/kg. The animals were killed 24 h later, and the cells of their bone marrow were examined for chromosomal abnormalities. The lowest dose of the benzilate did not give rise to any detectable chromosomal abnormalities; 1 mg/kg resulted in a 50% increase in the occurrence of chromosomal gaps and breaks in the bone marrow cells. The highest dose (10 mg/kg) induced a 325% increase in the incidence of gaps and breaks in chromosomes of bone marrow cells. The same dose of 3-quinuclidinyl benzilate had resulted in only a 107% increase in the incidence of chromosomal gaps and breaks; thus, diethylaminoethyl benzilate, despite its lower anticholinergic activity, was more toxic cytogenetically than 3-quinuclidinyl benzilate.

EFFECTS ON MAN

Davies (220) summarized the effects of diethylaminoethyl benzilate at various doses on human beings. Moderate doses--1-4 mg--characteristically produced a sense of divorce between outer reality and emotional reactions. Larger doses--4-8 mg--produced a more distinct detachment: muscles were felt to be relaxed and limbs were sensed as awkward, enlarged appendages with blunted sensations. The ability to concentrate was felt to be diminished, and thought processes were slowed. Mental vacuity was common, and reaction to external stimuli was slow. Continuity in thinking was lost, so that people who had taken doses in this range frequently said something like: "I have forgotten what I wanted to say." Complaints of mild palpitation and of dryness of the mouth might be made. The most common complaint among 110 patients treated with diethylaminoethyl benzilate was of a loss of ability to concentrate and a feeling of detachment from the people and objects in the immediate environment. All doses were given by mouth, the highest dose administered to this group of people being 16 mg/d for several months. No delayed effects or effects that persisted after benzilate administration was stopped were reported.

Raymond and Lucas (221) studied the effects of diethylaminoethyl benzilate on a group of 43 outpatients and 10 normal subjects. Patients given daily oral doses of the drug of 1 mg three times a day (t.i.d.) complained of no side effects. About half the patients who took 2 mg t.i.d. experienced side effects, particularly a sensation of limb heaviness. Most patients given 3 mg t.i.d. complained of side effects, including a feeling of heaviness or rubberiness of the legs, ataxia and clumsiness, difficulty in reading small print, poor ability to maintain continuity of thought or attention, giddiness, diarrhea, anxiety, and drowsiness. The 10 normal subjects were given single subcutaneous injections of 5 mg and then had their EEGs recorded. Five had markedly reduced alpha rhythms in their EEGs. Complaints made by these subjects after they had received the drug, in order of decreasing frequency, were weakness; headache, lightheadedness, and a feeling of heaviness of the limbs; drowsiness; and increased complexity and coloration of photic pattern and facial numbness. Complaints expressed only by some of the 10 subjects were dry mouth, difficulty in expressing thoughts, fear approaching panic, sensation that the floor was distant or seemed to slope, huskiness of voice, heaviness of the shoes, slight nausea, and sweating.

Kinross-Wright and Moyer (222) reported the results of giving diethylaminoethyl benzilate to 42 patients. They agreed with others that daily doses of 1 mg produced no effects that could be distinguished from those of a placebo. Side effects were infrequent with daily doses of less than 4 mg. With daily doses of 4-8 mg, there were complaints of dryness of the mouth, pupillary dilatation, slight palpitations, and difficulty in concentration. Daily doses of more than 8 mg resulted in such complaints by half the patients, with the addition in some of nausea, anorexia, and constipation. Patients given daily doses of 12 mg or more were affected also by feelings of heaviness of the limbs and of mental confusion, emotional lability and feelings of unreality, drowsiness, dizziness, and ataxia. One patient developed a maculopapular rash.

Vojtechovsky et al. (223) summarized their trials of administering doses of up to 75 mg of diethylaminoethyl benzilate to 17 healthy volunteers. The state of toxic psychosis--resembling a combination of psychoses due to atropine intoxication, to chronic alcoholic damage of the brain stem (Korsakov's syndrome), and to chronic alcoholic delirium--lasted for 4-6 h. They found no somatic complications after doses of up to 75 mg. These investigators suggested that the combination of anticholinergic activity and ability to inhibit monoamine oxidase (resulting in the demonstrated interference with the metabolism of serotonin and possibly of catecholamine transmitters as well) explains the phenomena of intoxication by this benzilate.

Rickels et al. (224) reported the findings in an experiment in which 52 patients were given tablets containing 1 mg of diethylaminoethyl benzilate and were instructed to take five tablets a day, but were allowed to decrease that to three tablets a day if it seemed desirable and, for short times, to two a day. The group of patients studied consisted of approximately equal numbers of psychiatric patients (mildly to moderately depressed neurotic outpatients) and patients seen in general medical practice. A placebo group (45 people) of similar composition received tablets of identical appearance and with the same instructions. The side effects experienced by the subjects were recorded 2 and 4 wk after beginning ingestion of the tablets. The six subjects who had taken a daily average of two or fewer tablets for a 2-wk period were excluded from the final compilations of data. Mean intakes of the two types of tablets were not stated, but would be expected to be about 4.5 tablets a day on the basis of reported intakes of similar tablets containing other drugs involved in the same experiment.

Of the 45 persons who took the placebo for 2 wk, 23 (51%)complained of side effects; 15 (33%) complained of sedation. Of the 52 persons who took the benzilate for 2 wk, 26 (50%) complained of side effects; 15 (29%) complained of sedation. Of the 30 persons who took the placebo for 4 wk, 17 (57%) reported side effects; 11 (37%) complained of sedation. Of the 26 persons who took the benzilate for 4 wk, 10 (38%) mentioned side effects; 4 (15%) complained of sedation. It is apparent that the persons who ingested diethylaminoethyl benzilate had no greater incidence of side effects than those who ingested the placebo.

Sidell (225) surveyed studies of the effects of 3-quinuclidinyl benzilate on human subjects carried out by Edgewood Arsenal or its contractors between 1960 and 1969. The initial studies were by and on four members of the research staff, who took the compound to become familiar with its effects, so that they could describe to potential volunteers the experiences that they might have as experimental subjects and could devise appropriate means for measuring functional impairment and safeguarding the subjects from physical harm. Sidell was able to identify 314 other subjects who had been given 3-quinuclidinyl benzilate at the Biomedical Laboratory at Edgewood Arsenal or at other sites. In addition, he reported that there had been 24 cases of accidental exposure to 3-quinuclidinyl benzilate among employees of Edgewood Arsenal. Of the 24, 14 had no effects other than mydriasis; four others complained of dry mouth, fatigue or sleepiness, photophobia, and inability to accommodate for near vision; six had difficulty in concentrating on a topic, slowed thought processes, and mild confusion. No long-term effects were reported.

In 1964 and 1969, field tests had been conducted with 3-quinuclidinyl benzilate. As a part of each test, eight volunteers were exposed to monitored aerosolized benzilate and were then assigned model military tasks to perform. A contractor had performed a study of the effect of exposure to this benzilate on the ability of trained pilots to fly airplanes, using the Link flight simulator and other appropriate measures of performance. The procedures used in 23 studies with 3-quinuclidinyl benzilate were outlined by Sidell (225), who also summarized the effects of the compound on man and stated that it is about 20 and 3 times as potent as atropine and scopolamine, respectively, in altering functions of the central nervous system. When equipotent doses were used, the effects of 3-quinuclidinyl benzilate lasted about 6 and 9 times as long as those of atropine and scopolamine, respectively.

Sidell's description of the effects induced by 3-quinuclidinyl benzilate is as follows:

> At low doses, the effects include a dry mouth, decreased gastric mobility, inhibition of sweating, an increase in heart rate, pupillary dilatation and loss of accommodation, mild sedation and mental slowing.
>
> At high doses these effects are intensified. There are marked disturbances of function at all levels of the central nervous system: motor coordination, attentiveness and control of thought and learning processes all decline. Confusion, restlessness, impairment in perception, interpretation, and memory span, poor judgment and deficient insight are all features of this syndrome. True hallucinations are present. If the dose is quite high the subject may become stuporous or even comatose for several hours.

Freedman (226) reported the findings of a research project designed to determine whether small amounts of 3-quinuclidinyl benzilate modify undesirably the ability of experienced pilots to perform the tasks involved in flying an airplane. The 18 pilots chosen to take part in the study, from a panel of 41 potential subjects, were 25-45 yr old (mean, 38 yr) and had flying times of 200-5,000 h (mean, 1,800 h).

A Link flight simulator and 14 tasks, including the Number Facility Test, were used to assess the competence of the subjects before and at intervals after intramuscular injection of 3-quinuclidinyl benzilate at 1-4 µg/kg. These tasks yielded a total of 35 items that were graded in cycles during each test period. Practically no effects followed doses of 0.5 and 1.0 ug/kg. After doses of 2 µg/kg, there were slight decrements in performance in some of the tasks, a definite increase in heart rate, a decrease in systolic blood pressure, and a slight increase in diastolic blood pressure. The subjects given 4 µg/kg all made lower scores on the performance tests and exhibited definite functional changes, including increased heart rate, dizziness, drowsiness, mydriasis, and difficulty in accommodating the eyes for near vision. Most of the subjects had increased diastolic blood pressure and complained of dryness of the mouth.

The tests associated with the Link flight simulator were the first to demonstrate effects of the 4-µg/kg dose; especially striking changes occurred in vigilance and in judgments of altitude, headings, air speed, and glide paths. These decrements appeared between 1 and 1.5 h after injection. By 3 h, half the subjects could not have completed a mission involving simply taking off and landing an airplane. None could have completed a moderately involved mission. The abilities of the pilots to perform the standard tasks presented to them decreased further up to the end of the observation period, 6 h 40 min after the benzilate had been administered.

In July 1962, it was estimated (227) that exposure of a human population to a Ct product of 3-quinuclidinyl benzilate of 118 mg.min/m^3 would result in incapacitation of 30% of the population, a Ct product of 170 mg.min/m^3 50% of the population, and a Ct product of 347 mg.min/m^3 84% of the population.

Ketchum (228) summarized the experiments with 3-quinuclidinyl benzilate performed between August 1960 and July 1963 with human volunteers. The report included an appendix, prepared by Claude McClure, Jr., on the chemistry and biochemistry of 3-quinuclidinyl benzilate. The outstanding information derived from this appendix is that the brain was the only organ of mice found to contain ^{3}H 48 h after intraperitoneal injection of [^{3}H]3-quinuclidinyl benzilate. Perfusion of this benzilate through the livers of rats had indicated that the liver destroyed the compound quickly, 90% of the compound having disappeared from the perfusate within 30 min and less than 0.2% of the compound being found unaltered in the bile. The products were not known. Several hypotheses of biochemical mechanisms of action of 3-quinuclidinyl benzilate were presented, but no firm support for any of them was available.

The main report concerned 290 exposures of 215 subjects other than staff members to 3-quinuclidinyl benzilate by intravenous injection (11.3%), intramuscular injection (39.3%), ingestion (12.4%), inhalation (21.7%), and application to the skin (14.8%).

Intravenous and intramuscular injections were considered to be equal in effectiveness, although changes occurred more rapidly after intravenous than after intramuscular injection. By ingestion, 3-quinuclidinyl benzilate was about 0.9 times as effective as it was by injection. Inhaled 3-quinuclidinyl benzilate was about 0.6 times as effective as it was by injection. Application to the skin in solution in benzyl alcohol resulted eventually (after 24-36 h) in a group of effects representative of the responses to an injection of about one-seventh the amount.

Estimates of the doses required to be inhaled (expressed as Ct, mg.min/m^3) to produce various effects were as follows: mild incapacitation (moderate dilatation of pupils and slight blurring of vision, minimal incoordination, some slowing of thought), 66-124; moderate incapacitation (hallucination, confusion, unorganized hyperactivity, incoherent speech, shortening of memory and attention span), 102-152; and severe incapacitation (stupor or coma, possibly preceded by a period of agitation, followed after 10-15 h by prolonged hallucination and inappropriate behavior), 110-165. The LD_{50} for a man was estimated to be 5.7-6.7 µg/kg.

Repeated daily exposures of volunteers to intramuscularly injected 3-quinuclidinyl benzilate at 1 µg/kg yielded no evidence of cumulative effects, but 2 µg/kg induced clear signs of cumulation. One of 12 volunteers given daily doses of 1-2 µg/kg appeared to develop tolerance.

Repetition of a second administration of 3-quinuclidinyl benzilate 2-3 wk after an initial dose induced a more rapid and more pronounced development of intoxication than the first dose; that suggested a sensitization mechanism in the response to the second dose.

Tetrahydroaminoacridine (THA) had been found to decrease heart rate when tachycardia was present, to increase secretion of saliva, to increase sweating and lower core temperature if it had become high, to reduce muscular rigidity, and to improve, at least temporarily, the mental status of an intoxicated subject. THA seemed not to antagonize sleepiness and might have promoted it. Physostigmine, given orally at 2-6 mg/h, caused nearly total remission of toxic effects within about 8 h after administration of the benzilate. Physostigmine seemed to be a more complete antagonist of the effects of 3-quinuclidinyl benzilate than was THA.

In 1965, the Directorate of Medical Research of the Chemical Research and Development Laboratories, Edgewood Arsenal, issued a booklet (229) on the management of casualties from 3-quinuclidinyl benzilate. The booklet gave the following sequence of effects to be expected in persons who had received large doses: in 1-4 h, tachycardia, dizziness, ataxia, vomiting, dry mouth, blurred vision, confusion, and sedation progressing to stupor; in 4-12 h, inability to respond effectively to the environment or to move about; and in 12-96 h, increasing activity, random unpredictable behavior, and gradual return to normal 48-96 h after exposure. The booklet recommended treatment of casualties with intramuscular injection of 3 mg of physostigmine salicylate, followed by another such dose 40 min later, if necessary. Maintenance by oral doses of 2-5 mg of physostigmine salicylate every 1-2 h was suggested. With improvement in mental status, the frequency and size of the maintenance doses can be reduced gradually. Heart rate was recommended as an indicator of the adequacy of therapy. The pulse should be between 70 and 80 beats/min when good control has been achieved. If the heart rate falls below 70 beats/min, the frequency or magnitude, or both, of the maintenance doses of physostigmine salicylate should be reduced, but the patient should be observed carefully for possible reversion to toxic delirium. Peripheral effects of physostigmine overdoses (profuse sweating, clammy skin, abdominal cramps, vomiting, muscular twitching, tremor, and weakness) may be antagonized by small doses of methylatropinium nitrate or, if that is not available, of atropine itself.

Kitzes and Vancil (230) reported the results of a study to determine the intramuscular dose of 3-quinuclidinyl benzilate that constitutes the minimal effective dose (MED) for a man, here defined as the smallest dose that produces a decrease of at least 25% in performance in the Number Facility Test. The effects on heart rate were also recorded. Thirteen male volunteers were given intramuscular injections of 3-quinuclidinyl benzilate hydrochloride at 2.3 or 2.7 μg/kg. One man given 2.7 μg/kg became confused and developed hallucinations. The most common complaints of the other volunteers were of dry mouth, blurred vision, and drowsiness. Several developed restlessness, an inability to sleep despite drowsiness, and anorexia. Of six men given 2.3 μg/kg, one had a decrease in performance in the Number Facility Test of more than 25%, and three had dilated pupils. This dose induced no tachycardia or hyperthermia. Of seven men given 2.7 μg/kg, five had decreased performance in the Number Facility Test of more than 25%, three had dilated pupils, two had heart rates greater than 100 beats/min, and one had a blood pressure above 140/90. There were no cases of hyperthermia (the report did not state the environmental conditions of the study). The mean heart rate after the larger dose was about 92 beats/min, whereas that after the smaller dose was about 79 beats/min.

Kitzes and Vancil estimated that the MED related to performance in the Number Facility Test was 2.54 μg/kg, with 95% confidence limits of 2.31 and 2.80 μg/kg, and that the MED for inducing changes in somatic functions (vision, heart rate, and blood pressure) was about 2.7 μg/kg.

Crowell (231) described experiments in which 27 male volunteers were given intramuscular L-2-alpha-tropinyl benzilate at 1.9-8.6 μg/kg after a previous unpublished estimate that the intravenous MED was about 3.8 μg/kg. This benzilate was found to have a quicker onset of effects after intramuscular injection and a briefer duration of action than 3-quinuclidinyl benzilate. Every subject who received a dose of 2.8 µg/kg or more experienced dryness of the mouth, blurred vision, and dizziness. Nearly all the subjects given 4.1 µg/kg or more complained of anorexia, nausea, weakness, confusion, hallucinations, and ataxia. Most expressed the illusion that their hands and feet were as red as blood. About one-third suffered headaches, and a few vomited. The intramuscular ED_{50} for inducing a decrement in performance in the Number Facility Test of at least 25% was about 3.1 µg/kg, that for increasing heart rate to at least 100 beats/min was about 5.6 µg/kg, and that for reducing performance in the Number Facility Test by at least 90% was about 5.9 µg/kg. For men given L-2-alpha-tropinyl benzilate at a mean dose of 6.1 µg/kg, the mean duration of severe effects of the compound was 2.5-3.1 h, and the mean time of onset for reduction of performance in the Number Facility Test by at least 90% was about 1 h. At 10 d after intramuscular injection of L-2-alpha-tropinyl benzilate, no evidence of any organic damage attributable to the compound could be found. Crowell's estimate of the incapacitating dose by intramuscular injection was 6.0 ± 0.75 ug/kg.

Kitzes et al. (154) reported the results of 40 experiments in which volunteers with body weights between 59.1 and 96.3 kg were given 3-quinuclidinyl benzilate at 2.0-8.0 µg/kg. These experiments revealed no clear relation between body weight or benzilate dose and performance in the Number Facility Test. As part of the same study, 10 experiments were performed with volunteers with body weights between 65.4 and 87.7 kg who were given L-2-alpha-tropinyl benzilate at 1.9-8.6 µg/kg. With this compound, there seemed to be no clear relation between dose and performance in the Number Facility Test, but there may have been a positive relationship between body weight and performance. When the range of weights of these subjects was divided in half, the upper half of the range included four experiments with men given a mean dose of 5.4 µg/kg and having a mean body weight of 85.3 kg, and the lower half included six experiments with men given a mean dose of 4.3 µg/kg and having a mean body weight of 69.7 kg. Despite the smaller mean dose of the benzilate received by the men in the lower half of the weight range, that half included all the men rated as being the most severely affected in performance in the Number Facility Test. With 3-quinuclidinyl benzilate, the two halves of the weight range contained close to the same proportions of severely affected men: 45.4% in the upper half and 48.3% in the lower half. Here, also, the mean doses for the two halves of the weight range were similar: 4.3 µg/kg for the upper half and 4.7 µg/kg for the lower half.

Ketchum et al. (189) reported the results of field tests in which two groups of four men each were exposed on different days to clouds of an aerosolized solution of 3-quinuclidinyl benzilate in chloroform. After exposure to the cloud, the subjects were rated on their performances in a variety of tests of physiologic and psychologic properties. Baseline performances in these tests had been recorded after 12-20 practice runs before exposure; the five best scores in the practice runs were averaged as the baseline scores. A dry run before the actual experiment incorporated all features of the experiment except the exposure; the scores on the test battery during the dry run did not deviate significantly from the baseline scores. The conclusions from the study were that the ED_{50} for incapacitation by 3-quinuclidinyl benzilate under field conditions is about 60 mg.min/m^3 for a man with a body weight of 75 kg and a minute volume of respiration of 15 L. Intramuscular injections of 2 or 3 mg of physostigmine salicylate were found to reverse the toxic delirium rapidly; repeated injections every 2 or 3 h or oral doses about one-third larger than the intramuscular doses, on the same schedule, restored and maintained acceptable performance in the test battery and in carrying out simulated guard duty of a post perimeter or simulated sentry duty in a foxhole under attack by an aggressor.

Craig et al. (232) reported the results of exposing 24 volunteers to high environmental temperatures at various times after they had been given intramuscular 3-quinuclidinyl benzilate at 3, 4, 5, or 6 µg/kg by intramuscular injection. Two series of exposure to heat were performed. In the first, four subjects were exposed to 41°C for 2 h on each of two days and six subjects were exposed to the same temperature during two 2-h periods on one day; on the third day after the first day of exposure to heat, all 10 subjects were given intramuscular injections of the benzilate during the first of two 2-h exposures to 41°C, separated by 3 h, followed by two similar exposures on the same schedule on the following day and by a single 2-h exposure of six men on the morning of the sixth day of the experiment (four of these men had another 2-h exposure to heat on the afternoon of the same day). In the second series, 14 men were exposed to 41°C for 8 h on day 1, to 52°C for 8 h on day 3, and to 41°C for 8 h on day 7, beginning 4 h after an injection of 3-quinuclidinyl benzilate.

The effects of the benzilate reached their maximum about 6 h after injection. At that time, the heart rate, skin temperature, rectal temperature, and inhibition of sweat secretion were increased in relation to the dose of benzilate administered. The highest rectal temperature was 38.3°C, an increase of less than 1°C above the mean normal temperature. The disturbance of regulation of body temperature by 3-quinuclidinyl benzilate under the condition of recumbent rest used in these experiments should not be hazardous to a healthy person. Physical activity during the exposure to heat might alter this general picture, although a previous study had found that the combination of 3-quinuclidinyl benzilate at 4 µg/kg, exercise, and an environmental temperature of 46°C produced only a slightly greater increase in rectal temperature than the exercise and environmental temperature without the benzilate. The inhibition of sweating by this benzilate regressed at about half the rate at which that induced by atropine regressed.

Klapper et al. (162) reported the results of followup studies on two men who had been given 3-quinuclidinyl benzilate and one who had been given L-2-alpha-tropinylbenzilate earlier. The first two had histories of intermittent hematuria before their service as volunteer subjects; at the times of the followup examinations, they had fewer than 10 red blood cells per high-power field in their urine. One of them reported that he had had a flashback of his experiences under the influence of the benzilate when drinking alcoholic beverages about 3 wk after exposure. There were no remarks about the man who had received the other benzilate.

Klapper et al. (163) examined the records of 24 volunteer subjects who had taken the MMPI test and then had been given intramuscular injections of 3-quinuclidinyl benzilate at 2.1 (six men), 2.4 (11 men), or 4.5 (seven men) µg/kg in an attempt to determine the factors of the MMPI that are best correlated with the response to the benzilate represented by performance in the Number Facility Test. The dose of 2.1 µg/kg produced a mean decrease in performance in the Number Facility Test of 27%, 2.4 µg/kg produced a mean decrease of 26%, and 4.5 µg/kg produced a mean decrease of 74%. The scales of the MMPI that were best correlated with the individual scores in the Number Facility Test were that for positive test-taking attitude for 2.4 µg/kg and that for hysteria for 4.5 µg/kg. There were no significant positive correlations for the lowest dose. There were significant negative correlations with the schizophrenia and the hypochondriasis scales after that dose, but these negative correlations were not reported for either of the larger doses.

Sidell et al. (164) found that S-(2-diisopropylaminoethyl)-ethylmethylphosphonothioate injected intravenously at 1.5-1.7 µg/kg after intramuscular injection of 3-quinuclidinyl benzilate at 6 µg/kg was effective in inducing rapid improvement in the scores of three volunteers in the Number Facility Test. This finding was similar to that reported earlier in subjects exposed to scopolamine.

Sidell (168) reported that four volunteer subjects given intramuscular injections of 3-quinuclidinyl benzilate at 7 ug/kg had their scores in the Number Facility Test improved dramatically by intramuscular injection of 7 or 11 mg of physostigmine salicylate followed by oral administrations of 24 or 12 mg, respectively, of the same drug during total periods of treatment of 43 and 42 h, respectively. Two other subjects given the same dose of the benzilate were treated entirely with oral administration of physostigmine in total doses of 80 and 211 mg during periods of treatment of 37 and 71 h, respectively. The scores in the Number Facility Test of the last two subjects were better maintained than those of the first two.

SUMMARY

3-Quinuclidinyl benzilate, the benzilic acid ester that has been studied the most by Edgewood Arsenal, has effects similar in general to those of the tropic acid esters, but more prolonged. Two other benzilic acid esters have similar actions. In part, the particularly long duration of the central action of 3-quinuclidinyl benzilate may be related to its greater affinity for nervous tissue, and especially for mitochondria within neuronal cells. In agreement with its especially strong adsorption on mitochondria, the subcellular organelles concerned principally with the supply of energy to the cell, this benzilate has been found to reduce the oxygen consumption by nerve cells stimulated in various ways. This and the other two benzilates on which some information was found have some activity as local anesthetic agents and as antihistaminic compounds, in addition to their activity as peripherally and centrally effective antagonists of cholinergic agonists. The only long-term toxicity study of a benzilic acid ester that was found was of the ester with diethylaminoethanol; no significant pathemas were found in rats during this lifetime feeding study, but the lifetimes of the rats fed the benzilate may have been shortened somewhat. Feeding of the ester with quaternized 3-quinuclidinol to rats for a year and gavaging dogs with this compound 5 d/wk for a year produced no significant pathemas.

Both cholinergic and anticholinesterase compounds antagonized the toxic actions of the benzilates. Although 3-quinuclidinyl benzilate has been found to have weak mutagenic activity for yeast cells in culture and to produce gaps and breaks in chromosomes of bone marrow cells, it has not been proved to produce heritable changes in mammals. Diethylaminoethyl benzilate was more toxic cytogenetically than 3-quinuclidinyl benzilate, despite the lower anticholinergic activity of the former compound.

ESTERS OF PHENYLCYCLOPENTYLGLYCOLIC ACID

This group of anticholinergic esters contains five compounds, the subject acid being esterified with the following alchohols: tropine, 3-quinuclidinol, 2-methyl-3-quinuclidinol, N-methyl-4-piperidinol, and a mixture of N-ethyl-3-piperidinol and N-ethyl-3-pyrrolidylmethanol. Of 20 reports on these compounds by personnel of Edgewood Arsenal and its contractors, 10 are on the ester with N-methyl-4-piperidinol, four on the ester with the mixture of N-ethyl-3-piperidinol and N-ethyl-3-pyrrolidylmethanol, and two each on the other three esters. The literature on the ester with N-methyl-4-piperidinol is entirely from Edgewood Arsenal, whereas more has been written about the ester with N-ethyl-3-piperidinol and N-ethyl-3-pyrrolidylmethanol (Ditran) by investigators other than those at Edgewood Arsenal than by the ones at that site. Most of the information about the other esters in this group originated at Edgewood Arsenal. The following discussion concentrates on the two most popular esters in this group.

The information on the lethal activities of the members of this group of esters is sparse. Even when reports dealing with lethality according to their titles were furnished by the Board on Toxicology and Environmental Health Hazards, the texts provided were brief and uninformative. An example is a report (233) entitled A Review of the Pharmacology and Toxicology of CAR 301,060. This compound is the ester of phenylcyclopentylglycolic acid and 2-methyl-3-quinuclidinol. The entire text reads:

> CAR 301,060, a BZ-like glycolate, is, on the basis of extensive pharmacological evaluation, a potent anticholinergic compound that displays marked behavioral

> and CNS effects. The compound, in most instances, is equal to or more potent than BZ, and has similar temporal parameters. It is exceptionally unique in regard to profound potency in the ACRBL-HAZLETON visual discrimination test. Extensive acute and subacute toxicological evaluation failed to reveal any extremely profound toxic or pathological effect in any major organ system at the doses tested. CAR 301,060 has a very substantial safety margin in all species tested.

Such pap is a waste of paper and time.

Other reports of that ilk (234-236) give a little useful information. The first of these (234) stated that repeated daily intravenous doses of the phenylcyclopentylglycolic acid ester of N-methyl-4-piperidinol produced mydriasis in monkeys, that doses of 0.1 mg/kg or more resulted in above-normal ratios of adrenal:body and testes:body weights, and that the highest dose administered, 1.0 mg/kg, resulted in unusually small livers. None of the organs mentioned was considered to have been harmed histologically. The second report (235) concerned the effects of the same compound in the dog. The lowest daily intravenous dose, 0.01 mg/kg, induced mydriasis; the two higher doses, 0.1 and 1.0 mg/kg, resulted in ataxia, decreased activity, and dryness of the mouth, in additon to mydriasis. They also induced significant effects on the performance of the dogs in a multiple-stimulus conditioned-avoidance test. The highest dose induced a statistically significant decrease in body weight. At necropsy, no significant effects that could be attributed to the agent were found. The third report in this group (236) described the effects of the phenylcyclopentylglycolic acid ester of 3-quinuclidinol on dogs. Repeated daily intravenous doses of 0.1 mg/kg produced maximal mydriasis that persisted throughout the study, decreased activity and ataxia, decreased concentration of the urine, and significant changes in the weights of the gonads and kidneys. The lower dose, 0.01 mg/kg, was not reported to have produced any effects other than mydriasis and decreased activity. The dogs that had been trained in the multiple-stimulus conditioned-avoidance test displayed a rapid development of tolerance to a daily dose of 0.1 mg/kg.

N-METHYL-4-PIPERIDINYL-(PHENYLCYCLOPENTYL)-GLYCOLATE (EA 3443)

N-Methyl-4-piperidinyl-(phenylcyclopentyl)-glycolate, also designated EA 3443, was found (193) to be effective both prophylactically and therapeutically in reducing the adsorption of 3-quinuclidinyl benzilate by the cat's motor cortex, caudate nucleus, and sensory cortex after intravenous injections of the benzilate and the glycolate. When given after the benzilate, EA 3443 reduced the retention of that compound by the medial and lateral geniculates. When it was injected first, EA 3443 reduced the adsorption of 3-quinuclidinyl benzilate by the hypothalamus and thalamus, in addition to the three parts of the brain first mentioned. Prophylactically administered EA 3443 decreased the adsorption of the benzilate by the hypophysis, liver, lungs, skeletal muscles, spleen, and sciatic nerve. When it was administered to cats after the benzilate, it reduced retention of the benzilate by skeletal muscles, lungs, sciatic nerve, pancreas, liver, spleen, and heart. When added to the fluids surrounding a segment of isolated guinea pig ileum, EA 3443 reduced almost to zero the response to acetylcholine. This glycolate had about the same activity in this regard as 3-quinuclidinyl benzilate, but the anticholinergic effect of the glycolate was the one more readily reversed by tetrahydroaminoacridine.

Kitzes and Ketchum (237) reported giving 39 volunteers intramuscular EA 3443 at 1.0-2.7 µg/kg. These doses induced no hallucinations or abnormal neurologic signs. Dry mouth, blurred vision, and drowsiness were the most common complaints. Mydriasis occurred to a mild degree. There was no consistent dose-response relationship for the heart rate, but that rate may have been decreased, rather than increased. The minimal effective dose for reducing performance in the Number Facility Test of half the subjects by at least 25% (MED_{50}) in this study was 1.21 µg/kg, with 95% confidence limits of 1.00 and 1.48 µg/kg. The time for onset of these minimal effects was about 8 h, and the duration of effects was about 4 h.

Kitzes et al. (154) found that 80% of a group with a mean body weight of 87.2 kg who had been given EA 3443 at a mean dose of 2.2 µg/kg suffered degradation of their performances in the Number Facility Test. Only 46.7% of a group with a mean body weight of 71.6 kg who had received a mean dose of 2.4 µg/kg suffered degradation of their performances in the same test. In the case of this compound, therefore, body size may have conditioned the response.

Hart and Balter (238) exposed an unstated number of volunteers to EA 3443 by application to an unidentified area of the skin at 60-120 µg/kg (mean, 68.5 µg/kg). The volunteers were tested with a modification of the Army General Classification Test before and at 7 wk after EA 3443 exposure. During the exposure, the Number Facility Test was used to determine whether the subjects experienced any degradation of their mental abilities. The criterion for an MED (two of five consecutive scores below 75% of the baseline score) was met by 71% of the exposed volunteers. A control group of 15 volunteers were tested and retested 20 d later without any known intervening exposure to a psychotoxic substance. The mean score of the control group at the second testing was 3.2% below that at the first testing. The second testing of the exposed group yielded a mean score for that group that was 2.4% above that at the first testing. Neither of these differences is significant.

Lavallee (50) reported that the minimal intravenous dose of EA 3443 that decreased performance by at least 25% in the Number Facility Test by half the members of a group of volunteers was 1.2 µg/kg. The corresponding incapacitating dose, the ID_{50}, was 3.1 µg/kg. That for 3-quinuclidinyl benzilate was 6.6 µg/kg. When EA 3443 was injected intravenously into dogs trained in a multiple-stimulus conditioned-avoidance test, a dose of 100 µg/kg decreased the number of correct responses to visual stimuli by about 28.2%. This decrement had been surpassed by that induced by 3-quinuclidinyl benzilate at 32 µg/kg. In monkeys trained in a visual-discrimination avoidance task, intravenous EA 3443 at up to 56 µg/kg had no significant effects on performance of the task. The same doses of 3-quinuclidinyl benzilate also had no significant effects on the responses of monkeys in this test. Lavallee concluded that the screening tests with dogs and monkeys were not satisfactory for predicting the relative potencies in man of the anticholinergic compounds tested.

Klapper et al. (163) found that the scale of the MMPI that was the best correlated with the degradation of performance of volunteers in the Number Facility Test after intramuscular doses of EA 3443 (six volunteers, 1.8 µg/kg; five volunteers, 2.4 µg/kg) was that for paranoia. The lowest negative correlation was that between the social introversion scale and the degradation of performance in the Number Facility Test. These statements were true for both doses of EA 3443; that is different from the situations with some of the other compounds studied in this way. With both atropine and 3-quinuclidinyl benzilate, the larger dose produced a degree of degradation of performance in the Number Facility Test that was best correlated with the score in a different scale of the MMPI from the one that yielded the best correlation with the lower dose of the same agent.

Armstrong et al. (239) used a test that required water-deprived rats to press four levers in a preset sequence to obtain water. After administration of EA 3443 by an unstated route, the ED_{50} for a minimal mydriatic effect was 37 µg/kg; that for reduction of the number of drops of water obtained within a given period was 53.2 µg/kg.

DITRAN (CS 4297)

In 1958, Abood et al. (240) reported initial studies of a group of esters of alkylated derivatives of piperidine, including a material that was thought to be N-ethyl-3-piperidyl-(phenylcyclopentyl)-glycolate. This substance was given the trivial name Ditran; it is known now to be a mixture of two compounds: the phenylcyclopentyl glycolates of N-ethyl-3-piperidinol and N-ethyl-3-pyrrolidylmethanol in the proportion 3:7. This substance was not as centrally active (producing hyperactivity in rats) as the methyl homologue, but was less lethal and more potent as a peripheral anticholinergic. Ditran was administered to more than 40 human volunteers by Ostfeld et al. (241) and was found to be actively hallucinogenic. Esters of 3-piperidyl alcohols were found to be much more hallucinogenic than corresponding esters of 4-piperidyl alcohols.

Abood et al. (242) compared 14 related esters of substituted piperidyl alcohols. They found that the phenylcyclopentyl and phenylcyclohexyl glycolates were more actively hallucinogenic in human subjects than diphenyl, phenylpropyl, or phenyl 2-thienyl glycolates. Esters of quaternized piperidinium alcohols were not hallucinogenic. A hydroxyl group on the carbon atom alpha to the carboxyl group of the acid was found to be essential for hallucinogenic activity. Ditran was found to have an intravenous LD_{50} for the rat of 19 mg/kg and one for the mouse of 44 mg/kg. The latter value may have been erroneous, inasmuch as Usdin and Efron (243) later stated that the intravenous LD_{50} for the mouse was 10 mg/kg. However, Usdin and Efron may have confused the dose given to human subjects by Abood *et al.*, which was 10 mg/man, with the figure for the LD_{50} for the mouse after intravenous injection. The value reported by Abood *et al.* by this route accords better with the LD_{50} after intraperitoneal injection in the compilation of Usdin and Efron than that stated by the latter authors. Usdin and Efron gave also intravenous LD_{50}s for the guinea pig and the rabbit of 45 and 12 mg/kg, respectively. Intraperitoneal injections of Ditran into mice and rats yielded LD_{50} estimates of 60 and 25 mg/kg, respectively. Little correlation between peripheral anticholinergic activity and psychotogenic potency was found by Abood *et al.* They reported that intraperitoneal injection of physostigmine at 0.5 mg/kg into rats 5 min before injection by the same route of Ditran at 25 mg/kg prevented mydriasis and lessened tachycardia and hyperemia of the tail and limbs, but had no visible effect on hyperactivity or on weakness and poor coordination of muscles.

Gershon and Olariu (244) assessed the ability of Tacrine to antagonize the actions of Ditran on human subjects and compared the effects of Ditran with those of mescaline, Sernyl, and LSD. The most common complaints of subjects given doses of Ditran by intramuscular injection or by mouth, or by both routes, were of hallucination, confusion, and impaired contact. Gershon and Olariu found that Tacrine was able to antagonize both the peripheral and CNS effects of Ditran in man, but was incapable of preventing those due to Sernyl. Succinate, which effectively reverses both the peripheral and central effects of Sernyl, had no activity against intoxication with Ditran. The authors gave the following typical sequence of signs and symptoms after intramuscular injection of 10-15 mg of Ditran: in 20-30 min, autonomic reactions (dryness of the mouth, slight tachycardia, flushed face, and muscular relaxation); in 45-60 min, central effects (some confusion, speech difficulty, decreased ability to concentrate, slight disorientation, occasional vertigo, and hallucinations); in 2-4 h, maximal central effects (hyperreflexia and ataxia); and in 5-8 h, abatement of symptoms and signs.

Biel *et al.* (245) reviewed the effects produced by a large group of anticholinergic compounds in an attempt to develop guidelines for the development of new psychotropic drugs and reached the following general conclusions: only the piperidyl and pyrrolidyl glycolates that were potent anticholinergic compounds exerted profound effects on CNS functions. However, peripheral anticholinergic potency did not endow a compound automatically with brain activity. Over 95% of a dose of Ditran is excreted in the urine within 2 h after its administration. The largest brain concentrations of several of these substances were in the hypothalamus and caudate nucleus, areas related intimately with emotion and mood. Psychotropic anticholinergic compounds had little effect on several enzyme systems. Ditran is a clinically effective antidepressant agent. An inhibitor of cholinesterases, tetrahydroaminoacridine, is capable of ameliorating the psychotic episode induced by anticholinergic psychotogens. Piperidine, trifluoperazine, and several other tranquilizers were effective in overcoming hyperactivity induced by anticholinergic compounds in rats.

Bell et al. (85) used dogs to study the psychotropic activity of Ditran and its antagonism. After developing a rating scale including six indexes of autonomically evoked activity, six of motor activity, and eight of central nervous system activity, they were able to present a graph demonstrating that the time courses of each of the major types of effect induced by intravenous Ditran at 0.5 mg/kg had a somewhat individual time course. Mydriasis, tachycardia, and decreased secretion of saliva were apparent within 3 min after injection. Between 3 and 6 min after injection, the pupil response to light became sluggish, and alertness and responsiveness to commands began to decrease. Within 12-15 min after injection, incoordination and ataxia had reached a plateau, and the pupil response to light usually had disappeared. Obstinate progression and disturbed behavior (whining or barking, pawing at the floor, and apparently chasing imaginary objects) became evident at 30-40 min after injection. By an hour after the injection, most of the dogs had collapsed. Prostration, from which the dogs could be roused temporarily by noise or handling, lasted for 15-120 min. The integrated total effect (obtained by summation of scores on the various parts of the rating scale) reached an approximate plateau at about 0.5 h after injection and remained there till about 2.5 h after injection. Approximately complete recovery from the effects of this dose on dogs did not occur until about 5 h after injection. Mydriasis was the last sign of effect by Ditran to disappear, often being evident for up to 8 h after injection.

Bell et al. (85) found that doses of physostigmine or neostigmine injected intravenously at 20 µg/kg 30 min after Ditran antagonized the effects on autonomic functions, but did not alter the central actions. Arecoline (150 µg/kg) restored salivation and heart rate to normal, but had no other detectable antagonistic actions when injected intravenously 30 min after Ditran. Tacrine at 1 mg/kg, injected intravenously 30 min after Ditran, abolished all effects of Ditran except mydriasis and decreased secretion of saliva. About 40% of the dogs so treated had relapses about 10 min after the dose of Tacrine; repetition of the dose of Tacrine induced complete and permanent recovery, except for persistent mydriasis. Some derivatives of glycolic acid (phenanthrylglycolic acids), p-phenylmandelic acid, and benzilic acid injected 30 or 60 min before Ditran shortened by up to 55% the duration of the intoxication due to Ditran, but did not alter the initial severity of its effects. These organic acids exerted less antagonism to intoxication with Ditran when they were administered therapeutically than when they were used prophylactically.

Bell and Gershon (86) reported that yohimbine antagonized the intoxicating actions of Ditran, but differed from Tacrine in this sort of activity, in that its antagonistic activity was less time-dependent than that of Tacrine. The latter had only slight antagonistic activity early in the intoxication and had greater activity when it was administered later after the Ditran, whereas yohimbine had more nearly equal antagonistic activities at various times after the dose of Ditran.

Brown et al. (246) gave 48 dogs intravenous Ditran at 0.5 mg/kg and 30 min later injections of saline, chlorpromazine, imipramine, or two unidentified substances, IN 1060 and W2045. IN 1060 is known also as cyprolidol, an antidepressant, but an equivalent for W2045 could not be found. Chlorpromazine and imipramine deepened the intoxication induced by Ditran, whereas IN 1060 and especially W2045 lightened the intoxication. When chlorpromazine and the two other compounds were fed to dogs daily for a month before the animals were given Ditran, chlorpromazine accentuated the psychotogenic action of Ditran, whereas IN 1060 and W2045 both attenuated the psychotogenic action of Ditran. After the month-long dosing with the last two antagonists, IN 1060 had a much greater effect on the central actions of Ditran than W2045, whereas the reverse had been true after single therapeutic administrations of the two drugs. For both compounds with both schedules of administration, the effects on the central actions of Ditran were greater than those on the motor and autonomic actions.

Banshchikov and Stoliarov (216) compared the effects of three of Biel's compounds--JB-318, JB-329 (Ditran), and JB-336--among themselves and with those of benactyzine and atropine. They also discussed the use of these compounds in the practice of medicine. Ditran had a much less prolonged action than JB-318, which was described as causing loquacity and hyperactivity with an elevated mood that persisted for days, weeks, or even months. In the few people who had been persuaded to take repeat doses of this compound, resistance to the hallucinogenic action developed, but the mydriatic effect persisted. The effects of all three of Biel's compounds were qualitatively similar and resembled those induced by atropine and benactyzine. Daily doses of Ditran were stated to lead to development of resistance to its actions: after 3 wk of daily intake, a dose of 20 mg/kg no longer resulted in mental disturbance. The posthallucinatory euphoriant action of Ditran has been applied to the treatment of patients in depressed states of mind, apparently with some success. Ditran has also been used in treating patients with neuroses and psychoses.

Albanus (42) reported that subcutaneous injection of Ditran at 0.5 mg/kg into 11 dogs resulted in the development of ataxia after a mean of 22 min, of obstinate progression after a mean of 31 min, and of complete loss of contact with the environment after a mean of 45 min. Obstinate progression disappeared about 230 min after the injection of Ditran, and ataxia after about 280 min.

Gershon and Angrist (247) summarized the research that had been done with Ditran and other anticholinergic psychotomimetic compounds and with possible antagonists of their psychotomimetic activities. Nothing particularly new was reported in this paper. Tacrine is regarded as the best overall antagonist.

Sayers and Burki (71) compared the effectivenesses of 13 psychoactive compounds, including Ditran, in four assays of anticholinergic potency: displacement of 3-quinuclidinyl benzilate from the particles in a homogenate of rat brain, antagonism of stimulation of isolated guinea pig ileum by acetylcholine, antagonism of induction of tremor by oxytremorine in mice, and production of pupil dilatation in mice. Ditran was graded first in the first two tests, third in the oxytremorine test, and second in the mydriasis test. For comparison, atropine was as good as Ditran in the second test, first in the last two tests, and second in the first test. Overall, therefore, Ditran was judged to be slightly less anticholinergic than atropine. The general conclusion was that none of these tests is satisfactory for predicting in vivo effects on brain functions.

Kligman and Copelan (156) reported that 16 volunteers had been used for studies with Ditran, but gave no information about the studies.

Ketchum *et al*. (24,165,166) gave 22 male volunteers intramuscular Ditran at 50-170 µg/kg and concluded that 150 ug/kg produced a syndrome of peripheral parasympatholytic effects, disturbances of basic neuroregulatory functions, and disruption of higher integrative functions that culminated in delirium characterized by restlessness, confused speech, hallucinations, and moment-to-moment variability. Twelve of these volunteers were treated with physostigmine and three with Tacrine after development of toxic psychosis. Both inhibitors of cholinesterases were found to be effective in overcoming changes in the Number Facility Test induced by Ditran.

3-QUINUCLIDINYL-(PHENYLCYCLOPENTYL)-GLYCOLATE (EA 3167)

The phenylcyclopentylglycolic acid ester of 3-quinuclidinol was included in the group of glycolic esters studied by Larsson *et al*. (192). They recorded its threshold dose for producing *a* psychotomimetic effect in the dog as 50 ug/kg. Five substances with threshold doses of 10 ug/kg had hydrogen bond strengths (expressed as log K, where K is the constant for the equilibrium between free and bonded CO groups in infrared spectra of the glycolates) of 0.8-1.2 (mean, 1.01). Two substances with threshold doses of 50 µg/kg, one being Ditran, had log K values of 0.80 and 0.93; two other substances with threshold doses of 0.5 mg/kg, one being tropinyl benzilate, had log K values of 0.92 and 1.2. It is evident that there is no correlation between the strength of the intramolecular hydrogen bond and the threshold dose for psychotomimetic effect.

Ketchum *et al*. (248) gave six volunteers intramuscular 3-quinuclidinyl-(phenylcyclopentyl)-glycolate by intramuscular injection at 2.4 µg/kg, seven at 2.9 µg/kg, and six at 3.4

µg/kg (the hydrochloride salt was used). Measurements and observations of the subjects were made hourly for 6 h, bihourly for another 6 h, and every 4 h thereafter until recovery was judged to be virtually complete. The MED_{50} was found to be 2.5 µg/kg, and the ID_{50} was estimated to be 4.1 µg/kg. With the latter dose, mild effects were to be expected after 2 h and severe ones after 5 h. Severe effects would be expected to last for about 96 h, and mild ones for about 240 h. Eighteen of the subjects were examined for evidence of organic changes, and 13 were given psychologic evaluations about 6 mo after they had received the compound.

Most subjects displayed some change from baseline performance and behavior when examined 2 wk after injection of the glycolate; these changes usually disappeared by the fourth week after the injection. The effects detected in these early followup examinations included increased irritability; mild impairment of memory, judgment, or abstraction; mental sluggishness with occasional confusion; nervousness; and tenseness. No positive evidence of organic change was found at 6 mo after the doses; there may have been a small increase in IQ at that time. The profiles in the MMPI of seven subjects who were tested 6 mo after exposure, as well as before exposure and a month after exposure, were close to the baseline profiles, although a month after exposure they had contained significant increases in the scores on the hypochondriasis, depression, hysteria, psychasthenia, schizophrenia, and mania scales. Intoxication with the glycolate was found to be treatable with physostigmine or Tacrine; because of the long duration of the effects in some subjects, doses of antagonists may have to be repeated for a rather long time.

L-2-ALPHA-TROPINYL-L-(PHENYLCYCLOPENTYL)-GLYCOLATE (226,086)

The actions of L-2-alpha-tropinyl-L-(phenylcyclopentyl)-glycolate on experimental animals have been described (188). This was found to be the most active and the safest (largest ratio of LD_{50} to MED_{50}) of the six possible isomers of tropinyl-(phenylcyclopentyl)glycolate by intravenous injection into mice. LD_{50}s by intravenous injections into male mice and rats were 61 ± 3 and 65 ± 3 mg/kg, respectively; the LD_{50}s by gavage in the same two species were 98 ± 21 and 170 ± 53 mg/kg. Depression of general motor activity and depression of respiratory activity were the principal effects observed in the two test species during the estimations of LD_{50}s. A range-finding test with intravenous injection of the glycolate into monkeys yielded an LD_{50} probably between 10 and 20 mg/kg. In the cat, the LD_{50} probably is greater than 20 mg/kg.

L-2-alpha-Tropinyl-L-(phenylcyclopentyl)-glycolate elicited mydriasis in the mouse after intravenous doses of 10 µg/kg or more. One estimate of the MED_{50} was 5.6 ± 1.5 mg/kg. Mydriasis became maximal after intravenous doses of about 0.3 mg/kg. Other effects that were evident after doses of 10-30 ug/kg were hyperactivity, hyperreactivity to touch, and motor deficits. Respiratory depression became evident after intravenous doses of 0.3-3.0 mg/kg. General activity became lessened and ptosis of eyelids appeared after doses of about 30-50 mg/kg. Mydriasis was the most persistent effect, which might last for 3-4 d after the highest doses mentioned above. With mydriasis as the indicator, this glycolate was about one-third as active when applied to the skin of the mouse as when injected subcutaneously (benzyl alcohol was a suitable solvent for application of the glycolate to the skin). Intravenous doses of about 50-100 µg/kg depressed a polysynaptic reflex (ipsilateral flexor reflex) in the anesthetized cat, but did not affect the patellar reflex. This glycolate was effective in antagonizing tremor in mice (induced by subcutaneous injection of tremorine at 10 mg/kg), in subcutaneous doses of 10-30 µg/kg whereas, by the same route of administration, doses of 12-16 µg/kg were effective in inducing mydriasis. The glycolate had a dose-related negative inotropic effect on the isolated, perfused rabbit heart, with only a slight negative chronotropic effect. In the intact anesthetized dog, it had dose-related negative effects on both heart and mean blood pressure. L-2-alpha-Tropinyl-L-(phenylcyclopentyl)-glycolate was active prophylactically against 2 LD_{50}s of TEPP (2.3 mg/kg intraperitoneally) in mice pretreated by intraperitoneal injection of benzoquinonium at 0.3 mg/kg (to prevent neuromuscular paralysis by TEPP), its intraperitoneal ED_{50} being 40 ug/kg.

Rats given five daily intravenous injections of L-2-alpha-tropinyl-L-(phenylcyclopentyl)-glycolate at 50 mg/kg during 8 d and rats given intravenous injections 5 d/wk for 1 mo at 10 mg/kg had no changes observable at necropsy, except decreases in the sizes of the spleen and the thymus in the animals given 50 mg/kg. Histologically, both groups of rats had increased numbers of granules in the Paneth cells in the crypts of Lieberkühn in the small intestine. Two of five rats given the largest dose were thought to have slight depletion of thymocytes.

Monkeys given daily intravenous injections of L-2-alpha-tropinyl-L-(phenylcyclopentyl)-glycolate at 0.4, 2.0, or 10 mg/kg 5 d/wk for a month had mydriasis with all doses. Tremors occurred in the animals given the two larger doses, and convulsions in those given the largest. Various extents of involution of the thymus and of increased granule formation in the Paneth cells were seen in animals given each of the doses. Fatty changes were seen in the livers of some of the monkeys given the two larger doses. One of five monkeys given 0.4 mg/kg died on the twelfth day, and one of five given 10 mg/kg died on the twentieth day. No specific cause of death was identified in either instance, but both animals had lost more weight than the means of the living members of their groups.

The actions of this glycolate in unanesthetized beagles were studied (249). Groups of four dogs were given daily intravenous injections at 0, 0.8, 4.0, and 20.0 mg/kg 5 d/wk for a month, receiving 21 doses during 29 d. Each group contained two males and two females. Three of the dogs given the highest dose were found dead on the fourteenth, seventeenth, and twenty-sixth days; the fourth dog in that group was moribund and was killed on the twentieth day. No animals from the other groups died during this study.

All dogs that received the glycolate had mydriasis and reduced activity, the mydriasis persisting even over weekends. In the high-dose group, panting, persistent anorexia, tremor, ataxia, prostration, fits of running movements of the legs (possibly accompanied by barking) in prostrate animals, and convulsions were seen commonly. These dogs lost an average of at least 23.5% of their body weight before death. The dogs of the groups given the low and medium doses of the glycolate lost 4.4% and 10.8%, respectively, of their original body weight during the administration of the glycolate. The heart rates of the dogs given the glycolate all increased during the first 15 min after administration of the first doses, whereas those of the control animals, given intravenous injections of saline, decreased slightly on the average. After the first weekend, after three injections during the previous week and before injection of another dose of the benzilate, the heart rates of the animals given the two higher doses were still above their baseline values. Baseline heart rates were not available for the dogs given the lowest dose, but the heart rates of two of the four dogs in this group on day 6 were above the highest baseline rate recorded for the other 12 dogs in the experiment. On the twenty-ninth day of the experiment, at 2 h after the last doses of saline or glycolate, the mean heart rates, in comparison with those measured at the same time after the first doses, for the three groups of dogs then alive were as follows: control, 9 beats/min below that on the first day; low dose, 29 beats/min below that on the first day; and medium dose, 43 beats/min below that on the first day.

The composition of the blood (blood chemistry and hematology) changed significantly only in the group of dogs given the largest dose of the glycolate. Final samples of blood were obtained on only two of the four dogs in this group; in both, the prothrombin time was prolonged, the total serum cholesterol concentration was very low, and the alkaline phosphatase activity in the serum was very high (70 and 198 K-A units/100 ml vs. 4-12 in baseline measurements). Both dogs had other evidence of interference with liver function: increases in the activity of glutamic-oxaloacetic transaminase and in the concentration of serum bilirubin in one, and decreases in the concentrations of albumin and total protein and in the ratio of albumin to globulin in the serum of the other. The first of these two animals had a concentration of serum urea nitrogen that was above the normal range, so there seemed to be some interference with renal function also. The most striking change in the hematologic picture in these two dogs was the complete eradication of eosinophilic polymorphonuclear cells from the blood. The total leukocyte counts and hematocrits were decreased in both dogs; one also had a moderate decrease in erythrocyte count.

Terminal samples of urine were obtained from the bladders of all four of the dogs in the group given the highest dose of the glycolate. Two were bloody, and three contained bilirubin, bile salts, and urobilin. All four samples contained albumin. The urine of the dog mentioned above as having increased serum urea nitrogen contained hyaline casts.

Electrocardiographic recordings from the dogs given the medium dose of L-2-alpha-tropinyl-L-(phenylcyclopentyl)-glycolate contained suggestions of deepening of the S wave and of elevation of the T wave. Because baseline recordings of the ECG had not been made for these animals, the significance of the apparent changes cannot be assessed.

The only significant pathemas found in the dogs at necropsy at the end of the experiment were in those given the highest doses of the glycolate. The livers of these dogs were pale yellow to orange and were coarsely lobular or swollen. Three of these dogs had bile staining of the sclerae and aortas. Two of the dogs had tarry material in their gastrointestinal tracts, reddish urine in their urinary bladders, and fatty bone marrow. Hemorrhages into the renal cortices were found in these two dogs. Microscopic examination of organs from these dogs revealed decreases in the size and number of the islets of Langerhans in one dog from each of the low-dose and high-dose groups and in two dogs from the medium-dose group. In the high-dose group, irritative and degenerative or necrotic changes were also found in the livers, kidneys, bone marrow, adrenals, testes, epididymides, and gastrointestinal tracts. The livers of all the dogs in this group were markedly fatty, the hepatocytes containing numerous small cytoplasmic vacuoles. Widespread vacuolation of the tubular epithelium in the cortical region of the kidney was seen in three dogs; epithelial crescents were seen in Bowman's spaces in the kidneys of two of these dogs. The bone marrow of all four dogs had shifted to an immature type, with increased numbers of blast forms of the granulocytic series.

2-METHYL-3-QUINUCLIDINYL-(PHENYLCYCLOPENTYL)-GLYCOLATE (301,060)

Hayes (250) reported the results of a study in which 29 men were given single intravenous doses of CAR 301,060, also called cis-2-methyl-3-quinuclidinyl-(phenylcyclopentyl)-glycolate, at 0.5-5.3 ug/kg . They were graded in performance in the Number Facility Test. Two subjects given 5.3 ug/kg became incapacitated (two consecutive scores in the Number Facility Test below 10% of baseline); the mean duration of significant effect was 73 h. These two subjects were disoriented, confused, restless, and less coordinated than normal, spoke disjointedly and in a slurred manner, had lapses of memory, and experienced visual hallucinations. One was episodically paranoid and mildly hostile to the medical personnel in attendance. Both were drowsy and lethargic. Their heart rates were increased markedly for more than 24 h after injection, but the peak of the tachycardia (+85%) occurred about 12 h after injection. This dose was the only one that resulted in pupil dilatation, by about 2 mm.

Doses of 2.0 and 2.7 ug/kg produced only slight and irregular increases in heart rate. The heart rate was increased in a dose-related manner when doses of 3.2 ug/kg and more were administered. The lowest dose that produced a significant decrease in the performance of one of four subjects in the Number Facility Test was 2.7 µg/kg; 3.2 µg/kg produced significant decrements in the performances of five of seven men, and 3.8 µg/kg caused significant decrements in the performances of six of eight subjects. Hayes concluded that the intravenous MED_{50} of this glycolate for man is about 3.0 µg/kg and that, after a dose of 3.2 µg/kg, a significant decrease in cognitive ability occurs about 5 h after injection and lasts for about 7 h.

ESTERS OF PHENYLISOPROPYLGLYCOLIC ACID

Two esters of phenylisopropylglycolic acid are among 26 anticholinergic substances studied by Edgewood Arsenal: N-methyl-4-piperidinyl-(phenylisopropyl)-glycolate (EA 3834) and 4-(1-methyl-1,2,3,6-tetrahydropyridinyl)-phenylisopropylglycolate (302,668).

N-METHYL-4-PIPERIDINYL-(PHENYLISOPROPYL)-GLYCOLATE (EA 3834)

Copelan (251) gave 16 volunteers EA 3834 at 1.0-2.7 µg/kg intravenously and administered the Number Facility Test to them at intervals thereafter. Two groups of two men given 1.0 or 1.4 µg/kg had their performances in the Number Facility Test unchanged. Some members of each of two groups of four men given 1.6 or 2.0 µg/kg had their performances reduced to below 75% of their baseline performances. Three of four men given 2.7 µg/kg had their performances reduced to below 75% of the baseline. The estimate of the MED_{50} was 1.96 µg/kg, with 95% confidence limits of 1.45 and 2.66 µg/kg. Significant change in performance in the Number Facility Test usually began at about 1.5 h at the highest dose administered (2.7 µg/kg), the mean time of onset of significant effect on performance was 1.9 h, and the duration of significant effect was 2.8 h.

Copelan described the effects of this agent as follows. At 3-15 min after injection, the subjects reported that they felt "high," "light-headed," or "dazed." At about this time, they were unsteady in walking and particularly in turning about. Talking among the subjects became quieter, and their speech was slightly muffled, but without significant slurring. A feeling of heaviness of the eyelids and a tendency to diplopia became evident at 10-15 min after injection. Sedation, with drowsiness and dozing, were noticeable at 15-30 min. One subject (dose group not stated) reported an illusion of undulating movement in the glass of a window. Two subjects (dose groups not identified) reported hallucinations of crawling ants and of moving strings or hairs on the floor, respectively. The same two subjects recognized that they were hallucinating. There was no confusion, significant disorientation, or delirium among the subjects in this study. dryness of the mouth, blurred vision, tachycardia, and mild reddening of the conjunctivae were noted occasionally and might appear within 15-30 min after injection. No abnormal changes in blood and urine were detected 24 and 48 h and 8 and 28 d after injection.

Averill et al. (252) summarized studies of the toxicity of EA 3834 in mice, rats, rabbits, cats, dogs, and monkeys. Table 3 gives the available single-dose LD$_{50}$s for both EA 3834 and 302,668. It is apparent that the latter is less lethal than the former in the species for which values on both compounds are available. The values for 302,668 come from Sim (253).

In addition to the LD$_{50}$ values stated above, Averill et al. summarized the results of studies in which graded doses of EA 3834 had been administered to various animals by a variety of routes. In mice, intravenous injections at less than 10 ug/kg induced mydriasis and those of 32-100 µg/kg induced unusual sensitivity to being touched, restlessness, and ataxia. Intramuscular doses of 100 mg/kg in the rat induced paralysis of the leg into which the compound was injected within 5 min after injection and jerking movements of the head, hyperpnea, and exophthalmos within about 15 min after injection. Intravenous doses of 0.7 µg/kg induced mydriasis in rabbits, and tachycardia followed doses of 10-100 µg/kg. Ataxia in this species appeared after intravenous doses of 3 mg/kg, and prostration and convulsion occurred after doses of 11-14 mg/kg. In the cat, mydriasis, decreased activity, increased heart rate, and ataxia all occurred after intravenous doses of 10-100 µg/kg.

The heart rate of the dog was increased and the performance of a conditioned-avoidance task was degraded by intravenous doses of 10-28 µg/kg. Doses of 12-37 µg/kg reduced physical activity, and those of 25-30 µg/kg induced ataxia. Prostration and convulsions occurred after doses of 10-20 mg/kg. Inhalation exposure of dogs to a Ct product of EA 3834 of 36 mg.min/m^3 resulted in increases in heart rate. Physical activity was reduced by exposure to Ct products of 40-50 mg.min/m^3. Mydriasis and ataxia followed exposure to a Ct product of 90 mg.min/m^3. A Ct product of 102 mg.min/m^3, with 95% confidence limits of 82 and 126 mg.min/m^3, caused 50% of the exposed dogs to fail in performance of the conditioned-avoidance task. In the monkey (species not stated), intravenous doses of 6 µg/kg induced mydriasis. Visual discrimination was reduced by doses of 7-31 µg/kg. Tachycardia and decreased activity followed doses of about 130 µg/kg. Prostration and convulsions were produced by doses of 14-16 mg/kg.

Sidell and Braun (254) discussed the results of an experiment in which two men were given EA 3834 by mouth at 7 µg/kg, four were given 10 µg/kg, three were given 14 µg/kg, and two were given 20 µg/kg. One man given 7 µg/kg and two given 10 µg/kg suffered severe effects (reduction of score in the Number Facility Test to below 25% of the baseline score). All the men given 14 and 20 ug/kg experienced severe effects.

Of those who experienced severe effects, the three with the lower range of doses had a mean dose of 9 µg/kg and experienced severe effects at about 2.3 h after drinking a solution of the agent in water. The five men with the higher range of doses had a mean dose of 16.4 µg/kg and experienced severe effects at about 1.4 h after ingestion. The two men given 7 µg/kg had a mean score of 47% of their baseline scores. The four men given 10 µg/kg had a mean score of 40.4%. The five men given a mean dose of 16.4 µg/kg had a mean score of 2.6%. The eight severely affected men had recovery times of 12-16 h (mean, 12.5 h). The two men given 10 µg/kg who did not suffer severe effects had recovery times of 6 and 10 h. The severe effects lasted 0.5-9.0 h; the mean duration of severe effects for the three men who took a mean dose of 9 µg/kg was 3.3 h, whereas that of the five men who took a mean dose of 16.4 µg/kg was 6.4 h.

Mild effects (reduction of score in the Number Facility Test by at least 25% of the baseline score) for all the severely affected subjects lasted 10-14.5 h (mean, 11.2 h), whereas those in the two men who took 10 µg/kg without developing severe effects lasted for 3 and 7.5 h. Mild effects in the two men given 10 µg/kg who never developed severe effects began 2.5 and 3 h after ingestion. In the eight men who developed severe effects, the mean onset time for mild effects was 1.3 h (95% confidence limits, 1.0 and 2.0 h).

McCarroll *et al.* (255) described a study with EA 3834 in which eight enlisted volunteers from the U.S. Army were divided into two four-man teams, with a ninth serving as the leader of both groups. The ninth man had been given intramuscular scopolamine at 23.5 µg/kg several weeks earlier to familiarize him with the effects that the others would experience later, but he was not given an anticholinergic substance during the actual study. The members of the two teams were given directions for performing a simulated military mission in the field and then practiced the mission for 3 d. On the fourth day, the teams went through the entire mission under the observation of two judges, who graded their performances on a number of items incorporated into the exercise. The exercise included capture and interrogation by a simulated aggressor. On the day of the test, intramuscular injections were given at 9 a.m., and the exercise was started immediately. It ended at 4 p.m. A battery of tests (heart rate, blood pressure, rectal temperature, Number Facility Test, and behavioral checklist) was administered to each volunteer at 9:30, 10, and 11 a.m., noon, and 1 and 4 p.m. The performance of volunteers on this day was graded by the same judges who had been present on the control day.

One four-man team received EA 3834 at 2 µg/kg (one man), 4 µg/kg (two men), and 8 µg/kg (one man), for a mean dose of 4.5 µg/kg. The second four-man team received 4 µg/kg (one man), 8 µg/kg (two men), and 12 µg/kg (one man), for a mean dose of 8 µg/kg. Both teams were considered to be composed of capable, effective soldiers on the control day.

On the test day, the team with the lower mean dose was judged to be marginally effective. The man given 8 µg/kg had to be removed from the exercise about 2 h after injection. The other members of that team stumbled and fell over minor impediments in the terrain, lost some of their gear, made serious errors in calculating an azimuth with compasses, were unable to remember simple directions or to repeat instructions given just before questioning, and were unable to operate a field telephone. The man given 8 µg/kg cooperated with the "enemy" interrogator and tried to follow his instructions without intellectual or physical resistance. The team that received the larger mean dose became totally ineffective within about an hour. The man given 12 µg/kg became incapacitated within about 30 min and was removed from the exercise. One of the men given 8 µg/kg became incapacitated about 75 min after injection, and the other became incapacitated about 141 min after injection. The man in this group given 4 µg/kg was not incapacitated, but the team leader had to prod him constantly to get him to accomplish the simplest tasks. For both groups, the correlation between performance in the Number Facility Test and in the various military tasks included in the scenario for the field exercise was good.

The volunteers evacuated from the field exercise were flushed and had hot, dry skins, parched lips and tongues, dilated pupils, tachycardia, and exaggerated deep tendon reflexes. After treatment with physostigmine salicylate (55 µg/kg intramuscularly), these volunteers were restored to lucidity within 20 min. After institution of maintenance doses of physostigmine salicylate by mouth, the subjects were able to return to the field exercise and to perform their duties capably. When the maintenance doses of physostigmine salicylate to the volunteer who had been given EA 3834 at 12 µg/kg were discontinued after four such doses, he became delirious again within about 3 h and remained in that state for 5 or 6 h. No abnormalities of renal and hepatic functions were detectable in any subject 24 h and 7 d after the injections of EA 3834.

Cucinell et al. (256) applied EA 3834 in a solution in ethanol to the skins of 11 volunteers on single occasions, of three volunteers on two occasions, and of two volunteers on three occasions. In all instances, the area of skin to which the solution was applied was about 9 cm^2 of the neck at the angle of the jaw. When the solvent had evaporated, the area of application was covered with a plastic cup or with Teflon taped to the skin. The contaminated skin was washed with alcohol 4 h after the application. Some of the volunteers were placed in a hot room at 40.5°C and 24% relative humidity with a wind speed of 0.5 m/s (slightly more than 1 mph); others remained under unspecified room conditions. Three of the volunteers who received EA 3834 more than once were exposed to it both in the hot room and in the regular laboratory rooms; the other two in this category were exposed only in the hot room. The amounts of the agent applied to the skin ranged from 0.1 to 5.0 mg in the hot room and from 2.0 to 4.0 mg in the regular laboratory rooms.

The hydrochloride of EA 3834 had no measurable effect on rate of sweating or performance in the Number Facility Test. When EA 3834 base was applied to the skin, the time to onset of inhibition of sweating (38-170 min) in the hot room was related inversely to the amount of the agent placed on the skin within the range 0.1-3.0 mg. The rate of change of the rate of sweat secretion once inhibition of that rate had begun was somewhat less variable (13-42 $mg/m^2.min^2$) within the same range of amounts of the agent, but was randomly distributed throughout the range. The two extreme values of the rate of change of the rate of sweating came from experiments in which sequential amounts of the agent near the middle of the range were applied to skin, the larger of the two amounts producing the smaller rate of change. Because the earliest time after application of EA 3834 to the skin at which the Number Facility Test was administered to the volunteers was 4 h, there is no factual basis for the assumption by Cucinell *et al*. that there is a constant relationship between the time of onset of inhibition of sweating and the extent of decrease in performance in the Number Facility Test. Their proposed expression for estimation of the systemic effect of contamination of skin with this agent depends on this spurious relationship and cannot be considered reliable.

4-(1-METHYL-1,2,3,6-TETRAHYDROPYRIDINYL)-PHENYLISOPROPYLGLYCOLATE (302,668)

Copelan (257) summarized experiments in which 20 volunteers were given 302,668 intravenously at 1.0-4.6 µg/kg and were tested at intervals for ability to perform in the Number Facility Test. Men given doses of 1.0, 1.4, and 2.0 µg/kg lost less than 25% of their baseline abilities to perform the additions. Of four men given 2.7 µg/kg, two lost at least 25% of their abilities. Of four men given 3.2 µg/kg, one lost 29% and another lost 24%. Four men given 3.8 µg/kg and two given 4.6 µg/kg had mean decrements of 41% and 50%, respectively, and all lost at least 25% of their abilities.

Karger (258) estimated the ID_{50} of 302,668 by giving 14 volunteers intravenous doses of 5.0-11.0 µg/kg and administering the Number Facility Test at intervals thereafter. Incapacitation was defined as the making of two consecutive scores below 10% of the baseline score. A clinical evaluation of incapacitation based on observation of the subjects was also made. The scores on the Number Facility Test yielded an ID_{50} of 10.1 µg/kg, with 95% confidence limits of 9.2 and 11.1 µg/kg. The clinical evaluations yielded an estimated ID_{50} of 9.5 µg/kg, with 95% confidence limits of 8.5 and 10.6 µg/kg.

As a second part of the same study, Karger gave six volunteers 302,668 intravenously at 10 µg/kg and administered physostigmine at various times. An intramuscular dose of 3 mg of physostigmine injected 10 min before administration of 302,668 attenuated the decrement in performance in the Number Facility Test for about 4.5 h after injection. At that time the performance of two unprotected subjects and of the two prophylactically protected subjects became essentially identical. When a dose of 3 mg of physostigmine was injected intramuscularly 10 min after intravenous injection of 302,668, there was rapid recovery from about 35% of baseline performance to about 92%, but performance in the Number Facility Test was down to about 20% of baseline by 2 h after the injection of 302,668. When the physostigmine was not injected until 45 min after the agent, performance in the Number Facility Test rose from zero to about 85% within about 45 min and to a peak of about 93% within 75 min. Performance in the test oscillated between 93% and 70% of baseline until about 3.75 h after injection of the agent and then drifted down to about 40% at 8 h. Thereafter, it improved gradually without further administration of physostigmine; thus, performance in the test had improved to about 60% of the baseline value by 10 h after initiation of intoxication. Karger concluded that the best results in maximally incapacitated persons would be obtained by giving repeated intramuscular doses of 2 or 3 mg of physostigmine at intervals of about 2 h.

Sidell et al. (259) estimated the incapacitating dose of 302,668 inhaled as an aerosol. Performance in the Number Facility Test was the criterion of incapacitation. Nineteen volunteers inhaled aerosols of the agent while seated and wearing an oronasal mask, so that the expired air could be collected and analyzed for unretained agent. Four ranges of retained dose of the agent were recognized: 1.99-7.60 μg/kg, five volunteers; 8.3-11.54 μg/kg, four volunteers; 12.36-12.95 μg/kg, six volunteers; and 13.05-18.77 μg/kg, four volunteers. The mean performances in the Number Facility Test of all groups fell below the level for minimal effect (score less than 75% of baseline score), but that for the last group was the only one that fell below the level for incapacitation (score less than 10% of baseline score). The mean performance of the last group was below the criterion for incapacitation from about 0.25 h to about 5.5 h after exposure. That group also underwent marked decreases in its scores on an Orthorator for near and far vision, in its ability to move pegs in a pegboard, and in its performance of a bend-twist-touch task, whereas the other three groups had comparatively minor changes in these four measurements.

The initial signs and symptoms induced in these volunteers consisted of dryness of the mouth, drowsiness, and ataxia; these were followed by hallucinations, marked confusion, poor coordination, restlessness, and prolonged impairment of accommodation for both near and far vision with the larger retained amounts. Retention of large amounts (15 μg/kg) sometimes resulted in fine fasciculations of muscles. One subject in each of the two highest ranges of retained agent vomited, and eight others, including subjects from all ranges of retained agent, complained of nausea. The heart rate was not altered significantly among the volunteers in the three groups with the lowest ranges of retention, but three of four subjects in the other group had heart rates above 100 beats/min. No consistent effects on blood pressure were observed. Pupil dilatation occurred in all groups; in some subjects among those with the higher retentions, mydriasis persisted for 72 h.

From the results of these exposures, Sidell et al. (259) calculated that the ICt_{50} for a man breathing 10 L/min would be 181 $mg.min/m^3$, with 95% confidence limits of 159 and 206 $mg.min/m^3$. For a man exercising moderately and breathing 15 L/min, the ICt_{50} would be 121 $mg.min/m^3$, with 95% confidence limits of 106 and 137 $mg.min/m^3$. The incapacitating dose of retained agent for 50% of exposed men, the IRD_{50}, was calculated to be 13.1 µg/kg, with 95% confidence limits of 11.1 and 15.3 µg/kg. Witn this retained dose, the time to onset and the duration of mild effects were expected to be 25 min and 12 h, respectively; the times for severe effects were predicted to be 30 min and 5 h, respectively.

Lavallee (50) collected and graphed data from human experiments that indicated that 302,668 at comparatively low doses (below 4 µg/kg) approached 3-quinuclidinyl benzilate in activity in decreasing performance in the Number Facility Test, but at higher doses (above 9 µg/kg) was only slightly more active than scopolamine. Extrapolation of Lavallee's graphs suggests that the doses of 302,668 and of scopolamine that would be able to lower performance in the Number Facility Test to zero would be identical.

Sim (253) summarized the body of research with 302,668, printed or unpublished, performed in 1964-1971. The first one-third of the report discussed the chemical and physical properties of 302,668. The last two-thirds summarized the studies performed to determine the actions of 302,668 and several other anticholinergic substances in animal and human subjects. As shown in Table I-3, 302,668 was most lethal to the rabbit and least to the monkey after intravenous injection. It was most mydriatic in the rat and least in the monkey. The species with the safest relation between the lethal dose and the mydriatic dose was the rat, followed by the dog. Species with much smaller values of the ratio of LD_{50} to ED_{50} were the mouse, the rabbit, the cat, and the monkey. Although 302,668 had approximately the same actions as other anticholinergic compounds, the only sort of activity at which it excelled was the inception of increased motor activity in the mouse. The ICt_{50} in humans had been found to be 121 $mg.min/m^3$, which was larger than those for 3-quinuclidinyl benzilate, EA 3580, and EA 3834. Amiton, diethyl-S-(diethylamino)-ethylphosphorothiolate, was mentioned as having been found to be a more potent and longer-lasting antagonist of the autonomic effects of 302,668 in cats than physostigmine.

ESTERS OF 1-PROPYNYLCYCLOPENTYLGLYCOLIC ACID

This group of glycolates includes two compounds: the esters of the subject acid with 3-quinuclidinol (302,212) and with N-methyl-4-piperidinol (302,196).

Copelan (260) gave 25 volunteers 302,212 intravenously at 1.0-4.6 ug/kg. The initial effect experienced by the subjects was a feeling of "light-headedness" or of mild "dizziness" that started 15-30 min after injection. Although this symptom was reported by the subjects to be especially noticeable when they were standing, it was not associated with orthostatic hypotension. Between 30 and 60 min after injection, the subjects became drowsy. They often fell asleep; when walking, they staggered mildly and often brushed against corridor walls. Speech became muffled and slow, but was not slurred. Slight impairment of logical thought and of performance in the Number Facility Test became evident about an hour after injection. The doses used in this study did not produce incapacitation. Peripheral effects were limited usually to dryness of the mouth and, after the higher doses, mild blurring of near vision. The compositions of blood and urine and the removal of Bromsulphalein from the blood were within normal limits at 24 and 48 h, 8 d, and 4 wk. The MED_{50} for inducing at least a 25% reduction in the score in the Number Facility Test was estimated to be 3.3 ug/kg. The latency of the effect with this dose was expected to be about 4 h, and the duration of the effect was estimated to be about 4 h. The latency of the effect changed little, if at all, as the dose was increased, but the duration of the effect increased irregularly, but rather definitely, after the larger doses.

Kligman and Copelan (156) stated that 25 subjects had been exposed to 302,212, but gave no information about the experiments. The 25 subjects probably were those used by Copelan (260).

Lavallee (50) collected data from studies of the effects of 302,196 on human subjects that enabled him to draw a line relating the effects on the score in the Number Facility Test to the logarithm of the dose. This ester was less effective than scopolamine in decreasing the ability to perform this test.

Sidell et al. (261) injected 302,196 into 10 volunteers intramuscularly at 14-54 µg/kg, bracketing the previously determined intravenous ID_{50} of 22.5 µg/kg. The ID_{50} after intramuscular injection (reduction in score in the Number Facility Test to not more than 10% of the baseline score) was calculated to be 28.9 ug/kg, with 95% confidence limits of 12.0 and 65.5 µg/kg. Therefore, this ester was nearly 80% as active by intramuscular as by intravenous injection. At the ID_{50}, the duration of severe effects was expected to be about 101 min and the total duration of effects to be about 263 min. The latency of onset of severe effects was expected to be only about 12 min after intramuscular injection. The ED_{50} of 302,196 to increase the heart rate by 30 beats/min was calculated to be 23.5 µg/kg. The maximal increase occurred at about 15 min, and the heart rate was close to the baseline value by 4-6 h after intramuscular injection. The mean blood pressure was increased slightly, with a time course similar to that of heart rate. Pupil dilatation was slower both in developing and in disappearing than other changes that followed intramuscular injection. No abnormalities of blood and urine were found at 24 h and 7 d after injection.

N-METHYL-4-PIPERIDYL-(PHENYLCYCLOBUTYL)-GLYCOLATE (EA 3580)

Mongrel dogs unselected for sex were given 20 daily intravenous injections of the hydrochloride of N-methyl-4-piperidyl-(phenylcyclobutyl)-glycolate (EA 3580) at 0.01, 0.1, or 1.0 mg/kg during a 28-d period (262). No detectable effects were observed with the lowest dose. Mydriasis, ptosis, dry mouth, decreased activity, ataxia, and weakness of limbs were observed after the two larger doses. Tolerance to the compound developed during the study.

A similar experiment was performed (263) with male rats. Mydriasis occurred at the low dose (0.01 mg/kg.d). Tremors occurred at the two higher doses. The rats given the two higher doses had their ratios of adrenal weight to body weight increased significantly. No quantitative information has been made available.

A similar experiment was performed with the free base in place of the hydrochloride (264). The same doses used in the two preceding experiments were administered daily, 5 d/wk, for about a month. All doses induced mydriasis. Fine body tremors also occurred. These two observable effects were the only ones mentioned. Their appearance was rapid after injection, their durations were "relatively short," and they were reproduced each day. The groups given the two higher doses had liver weights and ratios of liver weight to body weight that were significantly larger than those of the control rats.

These three estimations of subacute toxicity indicated that a modest number of repeated doses of EA 3580 in the form of the free base or the hydrochloride and in amounts of not more than 10 µg/kg should be safe, although possibly disturbing. Typical anticholinergic effects would be expected.

Aghajanian et al. (265) told of giving four volunteers EA 3580 intramuscularly at 1 µg/kg, seven at 1.4 µg/kg, four at 1.7 µg/kg, and five at 2.0 µg/kg. With the production of at least a 25% decrement in performance in the Number Facility Test as the criterion of a significant effect, the MED_{50} was calculated to be 1.47 µg/kg. After a dose of 2 ug/kg, significant decrement of performance extended from about 3 to about 12 h after injection; the maximal decrement after this dose (mean of four cases) was to about 60% of baseline performance. All subjects experienced drowsiness beginning about 1-2 h after injection and lasting for at least 6 h. Some of the subjects had mildly impaired intellectual functions, but no delirium or hallucinations. None of the subjects had tachycardia, but two subjects developed distinct bradycardia after unstated doses. There was no complete blockage of sweating, although sweating was reduced. Decreased ability to accommodate for near vision was observed in some subjects, and four subjects (with unstated doses) had spasms of accommodation.

A report (266) that presents the results of administering two unstated doses of EA 3580 by an unstated route to groups of four men has been made available. The subjects were familiarized with the problem-solving task, which involved deciding whether an array of seven letter and number symbols was the same as an array seen previously and then pressing one of two buttons to indicate whether the two arrays were the same or were different. Six days after a baseline session following the familiarization session, the subjects were given EA 3580 and were tested for ability to perform the task with the duration of presentation that had been found to yield a stable accuracy of 65-75% in the familiarization session and that had been used in the baseline session. About all that can be derived from the data presented is that EA 3580 produced a more marked decrease in the rate of responding than in the accuracy of the responses and that the effect was more marked under conditions of extinction (no reward for a correct response) than under those of reinforcement. One group of subjects was affected by EA 3580 more than the other, but the doses are unknown.

Crowell (267) presented an estimate of the incapacitating dose of EA 3580, in the form of the hydrochloride, for man, based on the results of intramuscular injections into 22 volunteers at 2.0-5.3 µg/kg. The criterion of incapacitation was reduction of the score in the Number Facility Test to not more than 10% of the baseline score for three consecutive scores. Doses of 2.0 and 2.7 µg/kg incapacitated none of nine subjects. One of five subjects given 3.8 µg/kg was incapacitated. All eight subjects given 4.5 and 5.3 µg/kg were incapacitated. The ID_{50} was calculated to be 3.9 µg/kg, with 95% confidence limits of 3.6 and 4.3 µg/kg. The duration of incapacitation after the ID_{50} was about 90 min; the duration of severe effects after that dose was estimated to be about 5 h. The time to onset of severe effects was about 2.5 h. No adverse effects of single doses of EA 3580 of the magnitude used in this study on the liver, kidneys, or blood-forming organs were detected. The effects on the volunteers were similar to those of other anticholinergic substances. By 24 h after administration of the largest dose (5.3 µg/kg), the subjects were able to care for themselves. Complete recovery did not occur until 28-52 h after injection, without apparent relation to the dose.

Kitzes _et al_. (154) found that two groups of men with average body weights of 83.1 and 66.7 kg that had received similar doses of EA 3580 (3.1 and 3.2 µg/kg, respectively) suffered significant decrements in their scores in the Number Facility Test in 45.5% and 53.8% of the cases, respectively. It seems probable that body weight played no important role in conditioning the response. This puts EA 3580 in a class with 3-quinuclidinyl benzilate and distinguishes it from EA 3443 and tropinyl benzilate. EA 3443 seemed to have a positive correlation between body weight and response, whereas tropinyl benzilate seemed to have a negative correlation between body weight and response.

Hart and Balter (238) found that volunteers exposed by the respiratory route to EA 3580 at 4.9-18.6 µg/kg and tested before and at intervals up to 5 wk after the exposure with a modified Army General Classification Test gave no evidence of persistent effects from the exposures.

Baker (268) compared the responses of volunteers to EA 3580 administered as a dose per man or as a dose per unit of body weight. Four indicators of effect were used: accommodation for near vision, arm-hand steadiness, dynamic flexibility, and manual dexterity. The general conclusion was that the use of the dose per unit of body weight may increase variance, rather than control for extraneous sources of variation, when the purpose of a study is to establish the effects of a substance itself. The use of multiple-regression equations was suggested as an approach to the establishment of definitive information on effects of chemicals.

Kitzes et al. (269) exposed 16 volunteers to an aerosolized 2% solution in acetone of EA 3580 in the form of the free base and subjected them to a battery of tests before and after exposure. The men were exposed to three ranges of concentration of the ester: seven were exposed to concentrations corresponding to doses of 4.9-5.9 µg/kg (mean, 5.4 µg/kg), six to doses of 6.4-13.4 µg/kg (mean, 9.1 µg/kg), and three to doses of 17.0-18.6 µg/kg (mean, 17.9 µg/kg). The lowest range of doses produced no hallucinations or hostile or irritable behavior. The middle range of doses produced hallucinations in four of the six subjects and mild hostile behavior in one. The highest range of doses produced hallucinations and hostile or irritable behavior in all three subjects. In one of these subjects, the degree of hostility induced was considered severe, whereas it was mild in the other two. All the subjects in the last group were incapacitated within 30 min after exposure. All became delirious, and one semicomatose. All the subjects in this group and two of those exposed to the middle range of doses were thought by physicians who were not privy to the details of the experiment to require treatment by injection and oral administration of physostigmine. The toxic delirium disappeared within about 15 min after the beginning of therapy.

The ID_{50} of aerosolized EA 3580 was calculated to be 8.4 µg/kg, with 95% confidence limits of 5.8 and 12.2 µg/kg. The time to onset of incapacitation was about 2 h, and spontaneous recovery from severe effects required about 9 h from their inception. The effects induced by inhalation of aerosolized EA 3580 were the same as those induced by intramuscular injection of this agent. To judge by comparison of the retained ID_{50} for the aerosolized material and the ID_{50} estimated by Crowell (267) for the intramuscularly injected compound, the inhaled compound seems to have only 46.4% of the activity of the injected compound.

Baker (270) added to the previously mentioned volunteers given fixed doses of EA 3580 in the form of its hydrochloride (268) 16 volunteers given intramuscular injections of the estimated ID_{50} for a subject of average weight; 16 other volunteers were control subjects. Nine measures of performance were used. Each volunteer was tested five times during a 25-h period after injection of agent or placebo. The indicators of effect of EA 3580, in order of decreasing sensitivity, were manual dexterity, accommodation for near vision, accommodation for distant vision, arm-hand steadiness, estimation of elapsed time, short-term memory, dynamic flexibility, and static strength.

Baker et al. (158), in a summary report, stated that performance in a series of tests had been shown to be correlated with various personal traits and that changes in these performances caused by EA 3580 were clearly differentially related to the particular subject being tested. If the personal traits of a subject were taken into account, a significant portion of the subject-related variance could be compensated. The opinion was expressed that in this way generalizations about effects of substances that would be independent of the special group of subjects used in the research could be generated.

Allen and Safer (271) compared the temporal characteristics of intoxication induced by intramuscular injections of 0.25 mg of EA 3580 in the hydrochloride form in men resting in the laboratory and in men considerably more active in connection with field testing. The subjects in both situations developed their initial deficits in performance in the Number Facility Test at the same rate, but the subjects in the field test experienced a somewhat smaller deficit and returned toward normal more rapidly. The duration of marked mental incapacitation among the subjects in the field test was about six-tenths that among those in the laboratory.

Lavallee (50) used log dose-effect graphs to demonstrate that EA 3580 was nearly as active as EA 3443 and considerably more active than 3-quinuclidinyl benzilate and four other anticholinergic substances in bringing about decrements in performance in the Number Facility Test. In contrast with the sequence of decreasing potency in human subjects of EA 3443, EA 3580, and 3-quinuclidinyl benzilate, the sequence in dogs performing a multiple-stimulus conditioned-avoidance task was 3-quinuclidinyl benzilate, EA 3443, and EA 3580. In monkeys performing a visual-discrimination and avoidance task, the sequence was EA 3580, EA 3443, and 3-quinuclidinyl benzilate. In a similar task involving visual discrimination and escape by monkeys, the sequence of effectiveness of the two numbered compounds was EA 3580 and EA 3443. It is clear that none of the screening tests with experimental animals had reproduced the sequence of potencies found in the human test. The author of the report suggested that groups of animals larger than the two of each species required for the screening tests might increase the reliability of the results obtained with laboratory animals.

Klapper et al. (163) found that, for six men given a mean EA 3580 dose of 1.8 μg/kg by intramuscular injection, the scale of the MMPI yielding the greatest correlation with their performances in the Number Facility Test was that for hysteria. For seven men given a mean dose of 3.5 ug/kg, the scale with the best correlation was that for mania. For the lower dose, the most negative correlation was with the paranoia scale; for the larger dose, the most negative correlation was with the depression scale.

Sidell et al. (164) found that intravenous injection of S-(diisopropylaminoethyl)-ethylmethylphosphonothioate into men intoxicated with sufficient EA 3580 to decrease performance in the Number Facility Test to 15% or less of their baseline values removed the decreases promptly and nearly completely. Oral administration of the same phosphonothioate was ineffective. The doses of the theraupetic compound were not stated; oral administration of a different dose of the phosphonothioate might be effective.

Armstrong et al. (239) stated that the ED_{50} for EA 3580 in decreasing the number of rewards obtained by rats trained in a sequential-response task was 37.1 μg/kg. The ED_{50} for producing the least detectable mydriasis in rats was 16-30 μg/kg.

MISCELLANEOUS ESTERS

302,282

Kligman and Copelan (156) reported that the 1-methyl-4-piperidyl ester of phenyl-(3-methylbut-1-yn-3-enyl)-glycolic acid (302,282) had been administered to 20 men. The results were reported by Copelan (272), who gave 24 volunteers intravenous doses of 1.0-6.5 µg/kg. Doses of 3.8 µg/kg and less of this agent had only slight effects on performance in the Number Facility Test by the 12 men given these doses. A dose of 4.3 µg/kg decreased the test scores to below 75% of the baseline scores for three of four men. A similar result followed doses of 5.4 µg/kg in four men. Doses of 6.5 µg/kg reduced the scores of all four men given them to below 75% of the baseline scores and reduced the score of one man to 9%. The mean score for this group was 30.5% of the baseline scores. The time to onset of a minimal effect after the MED_{50} of 4.4 µg/kg was estimated to be about 30 min, and the duration of the minimal effect was estimated to be about 30 h. The peak effect usually occurred about 2 h after injection.

The initial symptom was mild "dizziness." Heaviness of the eyelids was a part of the initial experience in many subjects. Mild unsteadiness on standing and walking began between 5 and 15 min after injection. At the same time, speech became muffled and slowed. Sedation, with drowsiness and dozing, started about 15-30 min after injection. About 30 min after injection, degradation of performance in the Number Facility Test began. A few subjects had visual hallucinations, and one had auditory hallucinations also.

Dryness of the mouth or throat was reported by some subjects given the lowest doses and by all subjects given doses of 3.8 µg/kg or more. Mild blurring of near vision and occasional urinary frequency began after doses in the upper part of the MED_{50} range, but never affected all men in a dosage group. Mydriasis was not apparent. Nausea was rare. Conjunctival reddening frequently became evident within the first hour after injection. No significant alterations in blood and urine were found at 24 and 48 h, 8 d, and 4 wk after injection.

Safer (273) gave eight volunteers intravenous doses of 302,282 at 7.5-13.5 µg/kg. Three of the subjects became anxious after injection and two of them became agitated, restless, and passively resistant to experimental procedures. One subject vomited and later developed mild myoclonus, hyperactive reflexes, and indications of effects on the pyramidal tracts (positive Chaddock's reflex and possibly positive Babinski's sign). One subject had a tactually detected arrhythmia, which was not detectable in a later recording of the ECG. All the subjects had increased alkaline phosphatase in their serum 24 h after injection; one subject whose baseline activity of alkaline phosphatase (12 K-A units) was at the upper limit of normal had that activity increase to 18.2 K-A units at 24 h after injection of 13.5 µg/kg. On the seventh day after injection, the activity of alkaline phosphatase in his serum was 11.5 K-A units. Alkaline phosphatase activity in one other subject rose from 7.9 to 12.4 K-A units at 24 h after injection and was 8.4 K-A units on the seventh day after injection. This subject also received a dose of 13.5 µg/kg. Two of the subjects had 5-10 white blood cells per high-power field on the seventh day after injection. No other abnormalities in blood and urine were reported.

Safer (273) concluded that the intravenous ID_{50} of 302,282 is 12.5 µg/kg, but that the compound should not be administered to human subjects at doses greater than 11.5 µg/kg because of the untoward changes mentioned above.

302,537

Brown (274) stated that the ester of 2-propenylcyclopentylglycolic acid with 3-quinuclidinol (302,537) is a potent antimuscarinic compound with profound effects on the central nervous system and with a potency comparable with that of EA 3580 and somewhat greater than that of 3-quinuclidinyl benzilate. The times to onset and extinction of effects were unusually short with this agent. It was judged to have no marked toxic or damage-producing effects in any organism or organ system studied. The doses and organ systems used were not stated. The organisms studied were the rat, the dog, and the monkey. The blood-forming organs, liver, and kidneys were said to have been normal after administration of the substance. Presumably, other organs also were examined for pathemas.

Leib (275) stated that he gave 302,537 intravenously at 1.0-7.5 µg/kg to 18 volunteers. However, Figure 1 of his report contains 21 points at doses of 1.0-5.6 µg/kg; no explanation for this discrepancy was given. Eight men given doses of 2.0 µg/kg or less had scores in the Number Facility Test greater than 75% of their baseline scores. One of three men given 2.8 µg/kg had his score in that test decreased to slightly below 75% of his baseline score. The 10 men given 4.0-5.6 µg/kg had their scores in the Number Facility Test reduced to below 75% of their baseline scores, but the lowest score represented in Figure 1 of the paper was 25%, for a subject given 4.8 µg/kg. The intravenous MED_{50} of 302,537 was estimated to be 3.3 µg/kg, with time to onset of minimal effects of 3 h. After a dose of 4.0 µg/kg, the time to onset of minimal effects was 2.6 h; but after a dose of 7.8 µg/kg, the time to onset of minimal effects was only 12 min. The duration of minimal effects after the largest dose was 43 h. Doses of 5.6 µg/kg and more were reported to cause hallucination and delirium accompanied by restlessness and anxiety. In the one graph for a man given a dose of 7.8 µg/kg, incapacitation, indicated by reduction of score in the Number Facility Test to below 10% of his baseline score, occurred about 90 min after the injection. Intramuscular injection of 3 mg of physostigmine about 7 h after the injection of 302,537 induced a marked, but temporary, improvement in the subject's performance in the Number Facility Test. Improvement lasted for about 2 h. Repetition of this dose hours later, after some spontaneous improvement had occurred, had much the same sort of effect as the first dose, but may have increased the rate of spontaneous recovery.

WIN2299

Fine et al. (276) found that the ester of cyclopentyl-2-thienylglycolic acid and diethylaminoethanol (WIN 2299) antagonized the muscarinic (lowering of blood pressure of the dog by intravenous injection of acetylcholine at 5-10 µg/kg), the nicotinic (increase in blood pressure by intravenous injection of acetylcholine at 300-400 µg/kg into an atropinized dog), and the central nervous system (production of large-voltage cortical discharges in unanesthetized curarized cats by Sarin) effects originated by cholinergic or anticholinesterase compounds. WIN2299 had intravenous and subcutaneous LD_{50}s for the mouse of 80 µg/kg and 460 mg/kg, respectively, according to information supplied by the Sterling-Winthrop Research Institute. Daily intramuscular doses of 29.3 µg/mouse, beginning on the day before irradiation was started and continuing until death, prolonged slightly (and more than atropine) survival of mice subjected to whole-body irradiation with 550 R of xradiation, according to Haley and Rhodes (277). The mortality was still 100%. These authors attributed the prolongation of life to diminution in activity of intestinal smooth muscle by the anticholinergic compound, with consequently slower leakage of infective and toxic entities into the body of the mouse from the intestinal lumen.

Pennes and Hoch (278) administered tablets of WIN 2299 to seven patients. Two patients given doses of 2 mg exhibited principally sedative effects. In addition, one of these people described visual illusions and "hypersensitivity to light and sound." Four patients given 6 mg experienced illusions, delusions, and feelings of unreality. The single patient given 10 mg had full-blown delirium, with complete disorientation and visual and auditory hallucinations. No information was supplied on the time courses of these effects.

Fink (279) supplied some information about the time course of effects of WIN 2299. He gave 2.0-3.2 mg intravenously and found that the subjects began to feel restless and excited by about 10,min after the injection. These initial effects might develop into tense fearfulness with visual illusions and delusions. Complaints of dryness of the mouth were elicited by questioning, but were not made spontaneously. The heart rate did not change unless or until excitement and hyperactivity developed. The effects of these doses of WIN 2299 lasted for 2-3 h.

Grob et al. (147) reported the effects of oral doses of 0.5-2.0 mg of WIN 2299 administered on seven occasions to two volunteers who weighed 46 kg (volunteer 1) and 77 kg (volunteer 2). A dose of 0.5 mg to volunteer 1 produced feelings of fullness and heaviness of the head, of being far away from the immediate environment, and of walking unsteadily with subjective and objective impressions of mental depression, with spells of crying. The same dose given to volunteer 2 produced only a sensation of heaviness of the head that began about 22 min after ingestion of the dose and that lasted for about an hour.

A dose of 1 mg to volunteer 1 produced the same effects as the smaller dose, but to a greater degree. There was no effect on heart rate, pupil diameter, or muscular strength. Effects began about 45 min after ingestion and ended about 5.5 h after ingestion. This dose had marked effects on volunteer 2, beginning 20-40 min after ingestion with feelings of slowed movement of the arms and the legs and of slowed speech and thought. These effects were followed by sensations of swelling of the tongue and face, dryness of the mouth, weakness of the limbs, mild giddiness, and marked mental depression. Objectively, there was obvious mental depression, with listlessness, apathy, asthenia, weeping, poor attention, slowed speech, thought, and response, impaired memory (particularly for events in the immediate past), mental clouding and confusion, inability to perform simple calculations, and drowsiness. The subject's grip strength did not change, but the ability to hold arms or hands raised from a position of rest decreased. There was slight objective unsteadiness in walking and slight asteriotaxic effect. There were visual illusions and a slightly increased activity of tendon reflexes. The heart rate and pupil diameter did not change.

A dose of 1 mg of WIN 2299 to volunteer 1 during a period of taking daily oral doses of 8 mg of TEPP produced the same effects as before, but time to onset was longer (90 min) and the effects were a little less severe than when TEPP was not being taken. On the basis of this experience, two additional volunteers (weights, 66 and 53 kg) who were receiving doses of 13-20 mg of TEPP during 5-10 h were given doses of 1 mg of WIN 2299. Ingestion of WIN 2299 had a slight inhibitory effect on mild symptoms induced by the TEPP, beginning after 20-40 min, but little or no effect on moderately severe symptoms. A second dose of WIN 2299 of the same size as the first taken 45 min after the first dose, or the ingestion of a single dose of 2 mg, had slight to moderate inhibitory activity on moderately severe symptoms induced by TEPP. These changes began about 30 min after ingestion of WIN 2299. The effects of WIN 2299 on the central nervous system were less severe in these subjects than in those who had not received TEPP. Atropine was a better antagonist of the effects induced by TEPP than WIN Z299.

NONESTER ANTICHOLINERGIC COMPOUNDS

Table I-4 contains the information that could be obtained on the single-dose lethal activities of the two substances in this group, benzetimide and mepiperphenidol. Information on the toxicities of these compounds was supplied by Janssen Pharmaceutica and Sharp and Dohme Institute for Medical Research, respectively. Benzetimide is 2-(N-benzyl-4-piperidyl)-2-phenylglutarimide, and mepiperphenidol is 1-(3-hydroxy-4-phenyl-5-methylhexyl)-1-methylpiperidinium bromide.

BENZETIMIDE

In addition to the information on single-dose LD_{50}s for benzetimide, Janssen Pharmaceutica supplied extensive information on subacute and chronic toxicities of this compound. Rats fed diets containing benzetimide or given daily subcutaneous doses 5 d/wk for 14 wk gained weight at a lower rate than control animals when they were fed a diet containing 10 mg/100 g, but not when they were fed one containing 1 mg/100 g or were given subcutaneous doses of 10 or 80 mg/kg. Daily subcutaneous injections of 1.25 mg/kg did not affect the rate of growth of the rats. In general, organ weights in these animals were below those in control rats. Brain weight was not reduced, however, so its relative weight increased. No pathemas or histopathologic changes were found consistently. Rats fed diets containing benzetimide at up to 10 mg/100 g for 21 mo had no unusual gross mortality due to the compound. Among the rats that died in the control group, males accounted for 37.5% of the deaths; among the animals fed diets containing benzetimide, males accounted for 52.2% of the deaths. Among those fed the diet containing the largest concentration of benzetimide, males accounted for 62.5% of the deaths.

Pregnant rats fed diets containing benzetimide at up to 20 mg/100 g on days 6-15 of pregnancy for one or three generations gave birth to litters of the same size as those borne by control rats and produced no abnormal offspring. Pregnant rats given daily subcutaneous doses of 0.63-160 mg/kg during the first 21 d of pregnancy experienced no striking effects at doses below 10 mg/kg. At 10 mg/kg, but not at 5 mg/kg, there was a decrease in the incidence of implantations to 60% of the females, compared with 90-92% in the controls. With daily doses of 40 mg/kg, one of 20 dams died before parturition, and the incidence of implantations decreased to 10% of the females. At the highest dose (160 mg/kg), three of 20 dams died, and no implantations were found. The weights of the offspring at birth were reduced slightly, but no abnormal offspring were reported.

Pregnant rabbits given benzetimide by gavage at 0.31 or 2.5 mg/kg on days 6-18 of pregnancy had increased numbers of dead and resorbed fetuses (28.5% vs. 7.7% in the control group), and one of 59 offspring born to the rabbits given the largest dose of benzetimide was deformed. These various experiments have demonstrated that benzetimide in the doses used gave no evidence of mutagenic or carcinogenic activity and only questionable evidence of teratogenic activity. High doses of the compound did interfere with reproductive processes.

Janssen and Niemegeers (280), on the basis of tests with prophylactic administration of benzetimide to rats given pilocarpine hydrochloride intravenously at 80 mg/kg, concluded that benzetimide had sufficient action on the central nervous system to justify its consideration as a theraupeutic agent in parkinsonism. They considered also that it was one of only three compounds, in a total of 23 anticholinergic substances examined, that were able at submydriatic doses to prevent lacrimation and salivation induced by pilocarpine.

Soudijn et al. (281) examined the binding of [^{3}H]benzetimide to subcellular fractions of the rat caudate nucleus and found both in vitro and in vivo that the (+) isomer was bound to the particles in a fraction composed of membranes and nerve endings to the extent of 4-7 times the (-) isomer. Fractions composed more nearly exclusively of membranes or of nerve endings bound the labeled material less extensively than the fraction that contained both structures. Binding of benzetimide in vitro was reduced markedly by atropine and by carbachol, but only slightly, if at all, by d-tubocurarine.

Beld and Ariens (282) measured the binding of benzetimide to microsomes from smooth muscle isolated from bovine tracheae and to particles in homogenates of bovine caudate nuclei. Plotting the amount of [^{3}H]benzetimide bound to the microsomes as a function of the concentration of [^{3}H]benzetimide in contact with them yielded a biphasic curve for (+)benzetimide, whereas that for (-)benzetimide was rectilinear. At a benzetimide concentration of about 6.3 nM, 9 times as much of the (+) isomer was bound to microsomes from vascular smooth muscle as of the (-) isomer. The particles in a homogenate of caudate nucleus bound about 5 times as much of the (+) isomer as of the (-) isomer. The saturable component of the material that bound (+)benzetimide had a higher affinity for the isomer than the nonsaturable component, which bound (-)benzetimide about as well as it bound the (+) isomer. When the microsomes were exposed to a large concentration of unlabeled (+)benzetimide before addition of [^{3}H]-labeled (+)benzetimide, binding of the labeled isomer seemed to be only to the unsaturable component. Atropine was found to bind to the same entities as benzetimide and to block binding of [^{3}H](+)benzetimide by the saturable component of the microsomal preparation.

Karger (283) gave 17 volunteers benzetimide intravenously at 1.0-10.0 µg/kg. He estimated that the MED_{50} for lowering the score in the Number Facility Test to 75% of the baseline value for 50% of a group of subjects was 8.9 µg/kg. All men who met this criterion for a minimal central action had difficulty in focusing, which lasted in one case for 24 h.

Klapper et al. (162) re-examined one subject who had received benzetimide at some unstated time in the past. No evidence of long-term effects was found.

MEPIPERPHENIDOL

White et al. (152) gave six volunteers intramuscular injections of 100-300 mg of mepiperphenidol (1.17-3.63 mg/kg) and found that all six had blurred vision and receding of the near point of vision. These effects had nearly disappeared by the following morning. Urinary urgency and an increase of 10.8 mm Hg in mean diastolic pressure were particularly striking effects. The mean systolic pressure increased by 5.9 mm Hg, and the mean heart rate by 45.3 beats/min. The mean breathing rate increased slightly. All the subjects complained of dryness of the mouth, and all but one of becoming drowsy and of feeling unsteady in walking. The dose of mepiperphenidol increased the effectiveness of photic driving of the EEG in half the subjects and reduced the alerting reaction in one of the six subjects. Two subjects complained of eye irritation.

Nine subjects given intramuscular injections of 1.95-6.5 mg of atropine sulfate (0.031-0.064 mg/kg) and 150-300 mg of mepiperphenidol (2.21-3.99 mg/kg) all had blurring of vision and marked receding of the near point of vision, which was still somewhat farther away than normal on the following morning (mean, 10.1 in. vs. 5.3 in.). The mean diastolic and systolic blood pressures increased by 10.0 and 5.6 mm Hg. respectively. The mean heart rate increased by 37.9 beats/min. All nine subjects complained of dryness of the mouth, and eight of drowsiness and ataxia. Four of these subjects experienced slight nausea. Three complained of eye irritation.

According to the data supplied by the Sharp and Dohme Institute for Medical Research, mepiperphenidol is one-tenth as active as atropine in antagonizing the actions of methacholine chloride on the dog. If this value is used to calculate an atropine-equivalent dose for the subjects who received both atropine and mepiperphenidol, those who received sufficient doses of the two substances to yield atropine-equivalent doses of more than 0.320 mg/kg had a four-in-seven chance of developing nausea. The four persons who experienced nausea in this group were three men and one woman; the remainder of these subjects given atropine-equivalent doses above the threshold dose were two men and one women. In the group given atropine alone, the man who was the only subject who complained of nausea had received atropine sulfate at 0.037 mg/kg. Eight other subjects (six men and two women) had been given larger doses of atropine; two of these subjects complained of headache (accompanied by dysmetria in one), and a third complained of vertigo. In the group given mepiperphenidol alone, a woman who had received an atropine-equivalent dose of 0.131 mg/kg complained of slight nausea. Four subjects in this group (three men and one woman) received larger doses of mepiperphenidol than the nauseated subject, without making any complaints.

On comparing the changes in the mean values for the objectively measured properties induced by the three drug regimens used by White *et al*. (152), those for the subjects given both atropine and mepiperphenidol were below those for the subjects given either compound alone for systolic and diastolic blood pressures and were above those for the subjects given either compound alone for the rate of respiration and the near point of vision. The change in the mean pupil diameter for the subjects given both compounds was the same as that due to mepiperphenidol alone and greater than that due to atropine alone, whereas the change in mean heart rate for the subjects given both agents was almost precisely halfway between those due to the individual compounds. On the basis of this analysis, atropine and mepiperphenidol appear to be mutually antagonistic in relation to the regulation of blood pressure, to be somewhat antagonistic in their actions on heart rate, to be slightly mutually antagonistic with respect to breathing rate and activity of the ciliary bodies in the eye, and to be neither antagonistic nor additive in action on the circular muscle of the iris.

Jager (94) found, in in vitro experiments, that the atropinase in rabbit serum was inhibited strongly by mepiperphenidol, whereas the atropinases in rabbit and guinea pig liver were comparatively resistant to inhibition by mepiperphenidol.

"TAB" MIXTURE

This mixture contained 50 mg of atropine sulfate, 210 mg of N-diethylaminoethyl benzilate (benactyzine), and 2 g of trimethylenebis(4-hydroxyiminomethylpyridinium)dibromide (TMB-4) in 100 ml of solution. Wiles and Ford (284) summarized the results of experiments in which rats, rabbits, dogs, and monkeys were given intramuscular injections of graded single doses of TAB. Rats of the two sexes were studied separately. All the rabbits used were males. Dogs and monkeys were unselected as to sex, but the groups of animals of these two species contained approximately equal numbers of the two sexes. The first sign of toxic actions seen in the rat, the rabbit, and the dog was ataxia; the first one seen in the monkey was ptosis of the eyelids.

Table I-5 summarizes the information obtained from the studies summarized by Wiles and Ford (284) and by Lee et al. (285). The intramuscular doses that resulted in the first signs of an effect in 50% of intact animals of the various species and in death of 50% are given. TAB appeared to be more lethal (by a factor of 1.67) to female rats than to male ones, although the threshold toxic doses for the two sexes were identical. The rat apparently was more resistant to intramuscular TAB than the other species tested, the male rat having an intramuscular LD_{50} nearly 4 times the mean of those for mice, rabbits, dogs, and monkeys. Dogs given 22.6 mg/kg had the activity of their serum glutamic-oxaloacetic transaminase (SGOT) increased during 5 h after injection by a mean of nearly 85%. Thereafter, SGOT fell; at 7 d after injection, it was not significantly different from the control value. Monkeys that died 2-3 d after injection had liver necrosis and deposition of collagen in the renal glomeruli. Monkeys had a series of alterations of the activity of SGOT similar to that reported for the dog, the mean activity 5 h after injection being increased by more than 118% above the baseline activity and decreasing slowly thereafter. Lee et al. reported that all mice that died did so within 2 d after a single injection of TAB, which these investigators concluded was no more lethal than the TMB-4 in the mixture.

Wiles and Ford (286) recorded the results of an experiment in which animals were given subcutaneous (rats) or intramuscular (dogs and monkeys) doses of TAB at 1.2-41.2 mg/kg 5 d/wk for 4 wk. Rats were hyperactive for about 1 h after each injection, whereas the dogs (beagles) and monkeys were less active after each injection than they had been before it. The response of the iris to illumination of the retina in the dog and the monkey became sluggish to absent, depending on the dose of TAB. Three rats, all from a group given doses of 12.9 mg/kg, died during the experiment; a male died after the fifth dose, and two females died after the last (twentieth) dose, but before they were to be subjected to necropsy. No definite cause for these deaths was found. No other animals used as subjects in the experiment died during the 4-wk study.

Holgate and Sidell (287) reported the results of an experiment in which three volunteers were given intramuscular injections of 2 ml of TAB and three other volunteers were given ones of 4 ml. By 9 min after injection, the mean performance in the Number Facility Test had been reduced to 81% (2 ml) or 59% (4 ml) of the baseline performance; by 5.5 h, performance was 98-99% of the baseline performance. The heart rate increased slightly (by 6.6% after 2 ml; by 44% after 4 ml) within the 0.5 h after injection; 6.5 h after either injection, it was below the original value. Systolic and diastolic blood pressures increased slightly after injection and then slowly fell to below the original values within 4.5-6.5 h after injection. The pupil diameter increased after both doses, and both near and far visual acuities decreased.

The most common complaints voiced by the subjects of the experiment were of dryness of the mouth, muffled and indistinct speech, dizziness, weakness of the arms and legs, diminution of the sense of balance, and slowing of reflex responses. Other complaints made less frequently included those of thirstiness, sleepiness, disorganized activity, distraction by noise, restlessness, uncoordinated mental activity, inability to focus the eyes, blurred vision, muscular twitching, weariness, discomfort in the throat during swallowing, malaise, inappropriate desire to laugh, nausea, weakness of the voice, and rubbery legs.

Sidell (288) described an experiment similar to that of Holgate and Sidell (287) in which four volunteers were given intramuscular injections of 2 ml of TAB solution and another four were given 4 ml. The smaller dose resulted in a decrease in the mean score in the Number Facility Test of 40% during the 15 min after injection, whereas the larger dose resulted in a decrease of 78% during the same time. The score returned to its preinjection value at a mean of 2.25 h after injection of the smaller dose, whereas 2-5 h were required for a return to the preinjection value after the larger dose (no mean time for the four volunteers given this dose was stated). Three of the four men given the larger dose experienced hallucinations.

The smaller of the doses administered by Sidell resulted in a slight increase in supine systolic blood pressure for 1-1.5 h after injection and a more marked and prolonged increase in supine diastolic blood pressure. Standing systolic blood pressure was 5-8 mm Hg below supine systolic blood pressure, whereas standing diastolic blood pressure was about the same as that in the supine position. Mean pupil diameter at 5 h after injection was about 0.5 mm greater than that before injection. After the larger dose of TAB, supine and standing systolic pressures were increased for about 0.75 h after the injection, and supine diastolic pressure was increased for about 3.5 h. Standing diastolic pressure was about the same as that before injection. At 5 h after injection, the mean diameter of the pupil was 2 mm greater than that before injection.

Sidell reported that the subjects who received the smaller dose of TAB had xerostomia, blurred vision, "dizziness" or light-headedness, drowsiness, slightly impaired coordination, and some difficulty with concentration and memory. These appeared 5-10 min after injection, and the subjects generally were free of symptoms by about 1 h after injection. One subject was markedly nauseated, vomited, and complained of a severe headache. The subjects who received the larger dose were grossly uncoordinated, one being unable to stand without assistance, in addition to having xerostomia and blurred vision. Three of the four men had hallucinations, poor attention spans, impaired ability to concentrate, and poor memory. The effects started within 5 min after injection, peaked at about 30 min, and had largely subsided by 2 h after injection. Two of the subjects, one from each dosage group, who had been made quite uncomfortable by the effects of TAB were treated with physostigmine 18 min after receiving TAB. In both cases, the toxic symptoms were ameliorated within 10-15 min after administration of the physostigmine.

Holgate and Sidell (289) summarized the effects of intramuscular injection of 2 ml of the TAB solution into three subjects who were exposed in a hot room at 35°C and a relative humidity of 90% for 1 h before the dose of TAB and for 2 h after the injection. Merely increasing the temperature and the humidity was not found to be sufficient to produce an increase in heart rate, so long as the subjects remained at rest. From the dose-response data reported earlier, a single dose of TAB failed to have much effect on this rate. Yet the combination of TAB and increased temperature and humidity greatly increased heart rate. Cognitive function was not significantly altered under the conditions of the control trials, although there was a tendency for the exercising subjects to have lower scores in the Number Facility Test than the group at rest. Comparison of the effects of a single dose of TAB in the dose-response study with those in this study indicates a synergistic relationship between TAB and thermal stress.

Holgate and Sidell (290) described an experiment in which eight volunteers were given intramuscular injections of 2 ml of the TAB solution after having trained in the performance of a lever-pressing task that involved both estimation of time and vigilance; four other volunteers were given intramuscular injections of 4 ml of the TAB solution after training in the same task. The 2-ml and the 4-ml doses induced increases in the mean heart rate of 30% and 90%, respectively. The maximal change in heart rate occurred 10-20 min after injection and lasted for about 4 h after the larger dose. After the smaller dose, the subjects continued to be able to estimate time reasonably well and to respond effectively to visual signals in the vigilance portion of the task. The larger dose of TAB resulted in failure by the subjects to initiate timing responses and to respond to visual signals in the vigilance part of the task. Auditory signals of the erroneous estimation of time and of failure to respond to the visual signals still evoked intermittent, but usually late, responses by these subjects. Holgate and Sidell concluded that the subjects could continue to function at a reduced level after the lower dose of TAB, but were virtually incapacitated after the larger dose.

It is apparent from the signs and symptoms of intoxication by TAB that the sublethal effects are due primarily to the anticholinergic components of the mixture. This impression is strengthened by the reported beneficial effects of administration of physostigmine to seriously intoxicated subjects. However, the data in Table 5 on the lethal effectiveness of the TAB mixture, in comparison with data in Tables 1 and 2, show that the LD_{50} of TAB is closer to that of TMB-4 than to that of either of its anticholinergic constituents. Lee _et al_. (285) estimated that the intramuscular single-dose LD_{50} of TMB-4 for male mice was 53.5 mg/kg, whereas that of TAB was 64.5 mg/kg. The corresponding figures for benactyzine hydrochloride and for atropine sulfate were 92.1 and 604 mg/kg, respectively. Mice that died after doses of TAB, of TMB-4, or of benactyzine hydrochloride did so after bouts of clonic and tonic convulsions, whereas those which died after doses of atropine sulfate were severely depressed and ataxic. Mice given benactyzine hydrochloride were very sensitive to tactile or auditory stimuli, which often originated seizures. This triggering effect was not reported for the mice given either TAB or TMB-4. The hindleg paralysis described as affecting only those mice given TAB may have resulted from a combination of the ataxic action of atropine and the blocking activity of TMB-4 at the neuromuscular junction.

DISCUSSION

The initial interest of the chemical defense establishment in anticholinergic compounds was related to their use in antagonizing the toxic actions of the nerve gases. The early research on the biologic actions of this group of compounds performed by Edgewood Arsenal and its contractors was directed toward identification of the limits within which atropine might be used advantageously as a prophylactic and therapeutic agent against intoxication by inhibitors of cholinesterases and toward determination of whether any synthetic anticholinergic compound, which might be more readily available than atropine during a conflict, had safety and efficacy at least equal to those of atropine. For example, the group of investigators at the Johns Hopkins Medical School (Abner McGehee Harvey, David Grob, Joseph Lilienthal, Jr., Richard Johns, John Harvey, etc.) studied several synthetic anticholinergic compounds that had seemed in preliminary tests with animals to have some superiority over atropine in antagonizing the toxic effects of inhibitors of cholinesterases. This was done in part by treating cholinergic crises, in patients with myasthenia gravis who were being given inhibitors of cholinesterase (DFP, TEPP, Sarin, and neostigmine) to increase their muscular strength, with the synthetic anticholinergic compounds and comparing the results of such treatment with those of similar administrations of atropine. Some normal volunteers also were used in this sort of research.

Unfortunately, none of the anticholinergic compounds tested as outlined above was found to have any superiority over atropine. The research with WIN 2299 summarized in one of the reports (147) included in the group submitted for this survey is an example of the work of the group at the Hopkins. Other groups doing research on anticholinergic compounds in the same era were those at Galesburg State Research Hospital (Harold Himwich and associates), Galesburg, Ill. (144,157), at the Montefiore Hospital (Yale Koskoff and associates), Pittsburgh, Pa. (158), and at the University of Illinois School of Medicine (Archer Gordon and associates), Chicago, Ill. (27,151).

After a decision had been made to seek a new type of chemical agent among compounds that had disruptive actions on the normal functions of the central nervous system, anticholinergic compounds became of interest again, because of the well-known activities of atropine and scopolamine in producing a temporary toxic, psychotomimetic state. Most of the reports in this survey belong to this later period, which started in about 1958. TAB, the last substance discussed in this survey, was intended to be a therapeutic agent in nerve-gas intoxication for self-administration by soldiers, as well as for use by medical personnel. It combined the peripheral and central anticholinergic actions of atropine and benactyzine with the principally peripheral cholinesterase-reactivating activity of trimedoxime (TMB-4). It was devised in approximately 1975 after Russian chemical protective kits captured by the Israelis were found to contain somewhat similar preparations.

Although the anticholinergic substances considered in this survey differ in chemical constitution, all seem to produce about the same effects. There is some variation in the balance between central and peripheral actions among these substances, the compounds containing quaternized amino functions having almost exclusively peripheral actions when moderate doses are administered. The anticholinergic compounds said to have been administered to volunteers, but not mentioned in the reports submitted for this survey, may reasonably be expected to have actions similar to those of the 24 that have been discussed, because of the similarity of actions among the 24.

Single-dose LD_{50}s for mice, rats, and rabbits have been found for 17 substances other than atropine that permit their lethal activities to be related to that of atropine. On the basis of atropine = 100, the lethal activities of single doses of the 18 substances fall in the following order of increasing potency: methylscopolammonium, scopolamine, d-hyoscyamine, atropine, methylatropinium, WIN 2299, caramiphen, EA 3834, 302,668, benzetimide, mepiperphenidol, 3-quinuclidinyl benzilate, benactyzine, L-2-alpha-tropinyl-L-(phenylcyclopentyl)-glycolate, TAB, tropinyl benzilate, Ditran, and L-2-alpha-tropinyl benzilate. No information on the single-dose lethalities of EA 3443, EA 3580, 302,196, 302,212, 302,282, 302,537, 3-quinuclidinyl-L-(phenylcyclopentyl)-glycolate, or 2-methyl-3-quinuclidinyl-L-(phenylcyclopentyl)-glycolate were provided or could be found in the literature. Such information should exist; in its absence, no precise judgment on the safety of these compounds can be made That is not to say that they are unsafe. The human studies carried out with these compounds indicated that none had persistent effects on the volunteers beyond several days after single doses.

Among the 13 former volunteers who had been given at least one of the compounds with which this report is concerned and who were re-examined by Klapper et al. (161) in 1970-1971, only one, of two who had received 3-quinuclidinyl benzilate, reported having experienced a flashback. The other abnormalities reported by or found in the nine former volunteers who had been given atropine, scopolamine, or 3-quinuclidinyl benzilate probably were unrelated to their serving as volunteers. The four men who had been given EA 3834, 302,668, L-2-alpha-tropinyl benzilate, or benzetimide apparently had no abnormalities.

Among the 16 compounds for which doses effective in producing some definite alteration of function were stated, data are available for 11 after intramuscular injection. The other five compounds were administered by intravenous injection. Because we have in no case an indication of the relative effectiveness of intramuscular and intravenous injections of these five agents, it is impossible to rank all 16 in order of relative effectiveness. Among the compounds administered to volunteers by intramuscular injection, the most active in inducing a degradation in normal function--most commonly assessed on the basis of the dose required to cause loss of at least 25% of the predose score in the Number Facility Test by 50% of a group of subjects--was EA 3443, followed fairly closely by EA 3580, EA 3834, and 3-quinuclidinyl benzilate. This ranking agrees with that by Lavallee (50), except for EA 3834, which was not included in that author's rating. Less active than 3-quinuclidinyl benzilate, in order of decreasing effectiveness, were mepiperphenidol, 3-quinuclidinyl-L-(phenylcyclopentyl)-glycolate, 2-methyl-3-quinuclidinyl-L-(phenylcyclopentyl)-glycolate, L-2-alpha-tropinyl benzilate, scopolamine, methylscopolammonium, and atropine.

Among the compounds administered by intravenous injection, the most active were 302,212 and 302,537. The intravenous doses of these two compounds required to induce a definite degradation in normal function in the volunteers were larger than the intramuscular dose of 2-methyl-3-quinuclidinyl-L-(phenylcyclopentyl)-glycolate required to satisfy the same criterion; thus, one surmises that these two compounds are probably weaker than the one or two agents that follow 2-methyl-3-quinuclidinyl-L-(phenylcyclopentyl)-glycolate in the list of compounds injected intramuscularly. The other compounds injected intravenously, in order of decreasing activity, are 302,668, 302,282, and benzetimide.

Regarding the 16 compounds for which incapacitating doses--usually calculated as the doses required to degrade the score in the Number Facility Test to not more than 10% of the predose score--were given, three routes of administration were used: intramuscular injection, intravenous injection, and ingestion. The most effective of the compounds injected intramuscularly was 3-quinuclidinyl benzilate, followed in order by EA 3443, EA 3580, 3-quinuclidinyl-L-(phenylcyclopentyl)-glycolate, L-2-alpha-tropinyl-L-(phenylcyclopentyl)-glycolate, L-2-alpha-tropinyl benzilate, scopolamine, 302,196, atropine, and Ditran.

The most actively incapacitating of the intravenously injected compounds was 302,212; its incapacitating dose was larger than that of 3-quinuclidinyl-L-(phenylcyclopentyl)-glycolate, so it probably fits into the series of compounds given by intramuscular injection two or more compounds below 3-quinuclidinyl-L-(phenylcyclopentyl)-glycolate.

Of the two compounds administered orally, EA 3834 was slightly more than twice as effective as WIN 2299. Its effective oral dose was about 44% greater than the ID_{50} for intramuscular L-2-alpha-tropinyl benzilate. EA 3834 apparently ranks fairly high in comparative ability to produce incapacitation, and WIN 2299 probably falls near the middle of the hierarchy of compounds with this type of activity.

Among the seven compounds injected intramuscularly for which times to onset of effect were stated, the most rapidly acting was 302,196, followed closely by scopolamine. Then followed in order L-2-alpha-tropinyl benzilate, 3-quinuclidinyl benzilate, 3-quinuclidinyl-L-(phenylcyclopentyl)-glycolate, EA 3580, and 2-methyl-3-quinuclidinyl-L-(phenylcyclopentyl)-glycolate. Among the intravenously injected compounds, the one with the fastest onset of action was 302,668, with a time to onset of 30 min, whereas intramuscularly injected scopolamine and L-2-alpha-tropinyl benzilate had times to onset of 15 and 60 min, respectively. WIN 2299 had a time to onset of 40 min after ingestion, whereas EA 3834, by the same route of administration, had one of 168 min. The two compounds other than 302,668 injected intravenously, 302,537 and 302,212, had times to onset of 90 and 240 min, respectively, so they probably fall in the middle and lower parts of the entire hierarchy.

Two of the 12 compounds for which durations of effects were stated are outstanding in this regard: 3-quinuclidinyl-L-(phenylcyclopentyl)-glycolate and 3-quinuclidinyl benzilate, with mean durations of incapacitation of 69 h. L-2-alpha-Tropinyl-L-(phenylcyclopentyl)-glycolate came in a weak third, with an estimated duration of incapacitation of 27 h. The other nine compounds for which this measure is available had durations of action between 90 min and 6 h. In order of increasing duration of effect they are EA 3580, 302,196, 2-methyl-3-quinuclidinyl-L-(phenylcyclopentyl)-glycolate, L-2-alpha-tropinyl benzilate, scopolamine, EA 3834, WIN 2299, 302,668, and 302,212.

Because of the particularly long durations of their effects, 3-quinuclidinyl benzilate and 3-quinuclidinyl-L-(phenylcyclopentyl)-glycolate (EA 3167) might be more hazardous to both corporeal and mental health than the other compounds on which reasonably complete information is available. However, 3-quinuclidinyl benzilate has one of the most favorable ratios of LD$_{50}$ for small rodents to ID$_{50}$ for humans. The corresponding ratio for EA 3167 cannot be stated, because no data on its LD$_{50}$ for experimental animals have been provided.

Of the five quinuclidinyl compounds on which information derived from experiments with human subjects was provided, we have measurements of lethality for experimental animals only for 3-quinuclidinyl benzilate. The only basis on which we can compare this group of compounds is effect on the volunteers. Of these five compounds, 3-quinuclidinyl benzilate is the most potent as an incapacitating agent, and it and EA 3167 (the next most potent of the five as an incapacitating agent) have particularly long durations of action, requiring 3-5 d for complete recovery. The other three compounds in the group have durations of incapacitation of about 2-6 h. As a group, these compounds are not particularly rapid inducers of whatever changes follow their administration; even after intravenous injection, 302,212 and 302,537 required 4 and 1.5 h, respectively, to induce incapacitation. After intramuscular injection, 3-quinuclidinyl benzilate, EA 3167, and 301,060 required 1.25, 2, and 5 h, respectively, to induce incapacitation. These long times contrast with 12 min for 302,196--*N*-methyl-4-piperidiylcyclopentyl-(1-propynyl)-glycolate. They probably reflect comparatively slow penetration into the brain and adsorption onto nerve cells there. The basis for the differences is not readily apparent.

In both animals and man, the earliest symptoms and signs that follow administration of any of the anticholinergic compounds are restlessness, a feeling of dryness of the mouth, dryness and flushing of the skin, and decreased motility of the gastrointestinal tract. The effects produced by these compounds can be categorized as effects on or through the central nervous system, effects on peripheral effectors, and effects on or through nucleotides.

The effects mediated through anticholinergic actions on the central nervous system include restlessness, shortened attentiveness and decreased ability to concentrate on a topic, sensation of dizziness or faintness, tiredness, lassitude, drowsiness, sensations of heaviness and of altered shape of the limbs, apprehension, flattening of the EEG and decrease in the predominant frequency, weakening or abolition of the visual alerting reaction in the EEG, increase in photic driving of the EEG, ataxia, nausea, mental slowing, underestimation of elapsed time, decreased sensitivity to pain, obstinate progression, vomiting, incoherent and extravagant ideation, xanthopsia and other chromatopsias, disturbed sleep, hyperreflexia with development of Babinski's sign, illusions, disorientation, hallucinations (predominantly visual), somnambulism, delirium (possibly violent), and coma with occasional convulsions.

Effects of anticholinergic compounds that may depend primarily on actions at neuroeffector junctions include dryness of mouth, difficulty in swallowing, hoarseness and dry cough, dryness of nasal mucosa and nasal stuffiness, dryness of conjunctivae, relaxation of smooth muscles of the intestinal tract with decreased peristalsis, vasodilatation in conjunctivae and sclerae, brightness and dryness of the eyes, mydriasis and cycloplegia, flushing of face and upper chest, increased body temperature during exercise or in a hot environment, temporary bradycardia (rarely, but possibly, with cardiac standstill), tachycardia, slightly decreased systolic blood pressure, increased diastolic blood pressure, headache or eyeache, difficult urination, dyspnea, atrial fibrillation, atrial ventricular dissociation, and ventricular fibrillation.

The only compound in this group whose fate in the body has been studied to a moderately satisfactory extent is atropine. Some information on the metabolic fates of 3-quinuclidinyl benzilate and of diethylaminoethyl benzilate is available (193, 219), but it does not account completely for all parts of the molecules. The binding of 3-quinuclidinyl benzilate to nerve cells (193,194) and to organelles from such cells (195) has been studied, but no detailed studies of its detoxification, pharmacokinetics, and molecular pharmacology have been found for use in this review. Little or no information on the biochemical aspects of the activities of the anticholinergic compounds surveyed here has been found. Whether such information exists in literature that has been withheld from consideration for this survey is unknown.

So far as the general impact of anticholinergic compounds on human health is concerned, the principal ill effect that has been attributed to long-term administration of these compounds is the induction or exacerbation of the dyskinesia that may occur after long-continued use of neuroleptic compounds. This condition is characterized by intermittent protrusion and rotation of the tongue and by chewing or biting movements of the jaws and mouth, in many cases with athetoid or choreiform movements of the limbs (292-296). The condition has been reproduced in animals, at least in part, by administration of neuroleptic drugs (297, 298). Scopolamine was found to increase the intensity of the experimental syndrome induced in mice by such drugs as methylphenidate and teflutixol (298,299). By analysis of electromyographic records from patients with tardive dyskinesia during administrations of various drugs and procedures, Mano and associates (300,301) concluded that the dyskinesia is induced by relative hyperfunction of dopaminergic neurons in the striatum established by excessive inactivation of cholinergic neurons by anticholinergic drugs.

There is abundant evidence that medication with anticholinergic drugs is contraindicated in human tardive dyskinesia (302-309). Burnett et al. (308) found that the improvement in tardive dyskinesia after discontinuance of the anticholinergic drug, when both an anticholinergic compound and a neuroleptic drug had been used to treat schizophrenia, was not due to any change in the patients' serum concentration of the neuroleptic compound, but must have been due to withdrawal of an effect by the parasympatholytic compound. Gerlach et al. (310) had advanced essentially the same idea in suggesting that centrally active anticholinergic drugs administered to patients with tardive dyskinesia increased the predominance of dopamine in the brain stem (established there by the induction by neuroleptic drugs of supersensitivity to that effector substance) by decreasing the effect of acetylcholine on striatal receptors and thereby disturbing the balance between cholinergic and dopaminergic effects.

Martys (311) investigated the drugs responsible for "certain" and "probable" reactions in 335 patients in a total of 817 patients treated during a period of years, finding that 51% of all the reactions were caused by drugs that had actions on the central nervous system. The most common of these reactions were drowsiness, nausea, dizziness, diarrhea, headache, and dry mouth. When the symptoms collected by investigators (311-314) who had used a variety of anticholinergic drugs for various purposes are arranged in order of decreasing frequency of specific complaints, the following list results: incoherence, disorientation, flushed facies, hallucination, mydriasis and cyclopegia, restlessness, hyperactivity, picking or plucking motions with the fingers, ataxia, motor incoordination, coma, tachycardia, confusion, dryness of the mouth, hyperreflexia, apprehension, fear, somnolence, fever (above 37.8°C), retrograde amnesia, toxic delirium, nausea, diarrhea, headache, nocturia, urinary retention, impotence, painful ejaculation, tremor, gastralgia, rash, itch, and vomiting.

Pfeiffer et al. (312) found that children generally were somewhat more sensitive to antimuscarinic drugs than adults; the root mean square of the difference in order numbers based on the frequencies of signs and symptoms of intoxication by 3-quinuclidinyl benzilate between adults and children was 2.66. Children had higher frequencies of occurrence than adults for 10 of the 15 effects counted.

Craig (315) analyzed the nature of death of 48 patients in a mental hospital whose deaths were attributed to asphyxia and found that 14 of the patients had choked to death. Because therapeutic regimens for psychotic patients commonly include combinations of neuroleptic, antimuscarinic, and tricyclic antidepressant drugs and because all these categories of drugs have the ability to induce sensitization of neuronal effectors to dopamine or to prevent access of acetylcholine to cholinergic neuronal effectors, or both, Craig and associates (316) undertook a further study of the association among alteration of the gag reflex, possible drug-induced neurologic syndromes, and the administration of drugs to chronically hospitalized psychiatric patients.

Of the 58 patients in the study (316), 34 were considered to have normal gag reflexes, nine to have variable responses to touching the posterior pharngeal wall, and 15 to have definitely impaired gag reflexes. The last two groups were combined for the analysis of drug use. All 58 patients had been receiving neuroleptics and antiparkinsonism drugs; 12

(35%) of the 34 patients with normal gag reflexes had received anticholinergic drugs, whereas 15 (62%) of the 24 patients with variable or impaired gag reflexes had received anticholinergic drugs. In the group with normal gag reflexes, only 14 (41%) had facial-oral dyskinesia, and only 19 (55%) had dyskinesia of some part of the body. In contrast, 19 (79%) of the patients with variable or impaired gag reflexes had facial-oral dyskinesia, and 20 (83%) had dyskinesia of some part of the body. Craig et al. concluded that patients with tardive dyskinesia, and particularly those receiving anticholinergic drugs, are at high risk of having impaired gag reflexes and, consequently, of having swallowing impairments that increase their risk of death by asphyxia associated with entrance of food or other foreign material into the larynx.

Injections of atropine and hyoscine into chicken eggs on the fourth day of incubation have resulted (291) in increased rates of death of the embryos (e.g., 33% living on the twelfth day of incubation vs. 91% alive in eggs into which saline was injected) and of malformation (e.g., 33% of those alive on the twelfth day of incubation vs. 0% occurrence in the control eggs). Gastroschisis was the most common malformation, accounting for about 71% of the abnormalities found in the embryos. Despite this finding in the closed environment of the egg, there have been few findings suggestive of a teratogenic effect of anticholinergic compounds in mammals. One report (317) described an acranial and anencephalic fetus with complete atelectasis of the lungs, hemorrhage into the lungs, and hypoplasia of the adrenals born to a woman who had been treated during pregnancy for a duodenal ulcer with 10 mg of oxyphencyclamine two or three times a day. (As the saying is, one swallow does not make a summer; or, to corrupt Virgil's imperative, ab uno non disce omnes.)

The survey by the General Practitioner Research Group (136) found only one among 57 instances of reproductive accidents in the group of 661 women who had taken drugs of some sort during the first trimester of pregnancy that could possibly be related to an anticholinergic drug. It should be noted, however, that two-thirds of the accidents of reproduction found in this survey were related to the use of antihistaminic drugs, many of which have some anticholinergic activity. Although anticholinergic drugs are useful in ameliorating the results of lack of inhibition of contractions of the detrusor muscles of the bladder, the danger of causing the development of hydronephrosis by using such drugs in cases with obstruction of urinary outflow from the bladder has been noted (318).

With respect specifically to 3-quinuclidinyl benzilate, several drugs containing the quinuclidine ring, including quinidine, are in more or less regular use. The closest relative to 3-quinuclidinyl benzilate is clidinium bromide, which is a component of Librax. This preparation consists of one-third clidinium bromide and two-thirds chlordiazepoxide and is administered usually in unit doses of 7.5 mg, one or two such doses being prescribed three or four times a day. Several papers (319-323) have reported side effects experienced by patients advised to take Librax, usually for ulcers of the gastrointestinal tract. The most common side effects of use of this mixture were dry mouth and constipation. Other fairly common effects were drowsiness, headache, gastritis, nausea, and vomiting. Also mentioned in these papers were blurred vision, ataxia, tachycardia, and difficult micturition. Females were reported (319) to develop higher heart rates and greater tendencies to constipation than males; males had a greater incidence of difficult micturition than females. There was one report (321) of petechiae and thrombocytopenia in a woman who developed these conditions while taking one unit dose of Librax three times a day. After discontinuance of Librax, her platelet count, which had fallen to 12,000/mm^3, became normal (200,000-500,000/mm^3) within 5 d.

Although *N*-benzylnortropinyl benzilate was found (324) to be more *potently* antimuscarimic than 3-quinuclidinyl benzilate, the latter compound produced greater disruption of the behavior of mice and rats than any of eight other antimuscarinic compounds tested, including *N*-benzylnortropinyl benzilate.

Although one of the *two* former subjects who had been given 3-quinuclidinyl benzilate re-examined by Klapper *et al.* (162) was the only former subject to have had an *apparent* flashback, a conclusion that 50% of these subjects experienced flashbacks probably would be highly erroneous. The available evidence from studies with both animals and man is that recovery from intoxication with this benzilate, although it occurs slowly, is complete. Mice and guinea pigs given five subcutaneous doses per week of 3-quinuclidinyl benzilate at 150 ug/kg for 3 wk (total maximal dose, 2.25 mg/kg) were reported (184) to have developed no lasting signs of toxicity. The experience in experiments with both laboratory animals and man has been that generally the mydriatic and cycloplegic effects on the intrinsic smooth muscles of the eye are the ones that persist longest after doses of 3-quinuclidinyl benzilate. The cytotoxic actions of this benzilate (206,207) do not seem to have been reflected in definite heritable mutagenic effects.

The general conclusion of this survey is that there is no firm evidence that any compound surveyed carries a direct hazard of persistent diminution of human health and normal function in the doses used by Edgewood Arsenal and its contractors or an indirect hazard of abnormality of structure or function in offspring of the former volunteers. However, the cryptic activities of these compounds need to be studied much more intensively than they have been.

TABLE I-1

Representative LD_{50}s of Tropic Acid Esters in Laboratory Animals

	LD_{50}s, mg/kg											
	Mouse				Rat				Guinea Pig			Rabbit
Ester	P.O.	S.C.	I.P.	I.V.	P.O.	S.C.	I.P.	I.V.	P.O.	I.P.	I.V.	I.M.
Atropine	693	695	236	74	773	1,750	256	41	1,100	277	163	588
d-Hyoscyamine	--	--	--	81	--	--	--	--	--	--	--	--
Scopolamine	--	1,700	119	163	1,270	3,800	--	--	--	--	--	--
Methylatropinium	1,320	--	110	--	1,605	1,800	--	--	--	--	--	--
Methylscopolammonium	3,200	--	--	3,800	2,480	2,060	126	--	--	--	--	--

TABLE I-2

Representative LD_{50}s of Benzilic Acid Esters in Laboratory Animals

		LD_{50}s, mg/kg			
Animal	Route of Administration	3-Quinuclidinyl Benzilate	Tropinyl Benzilate	L-2-alpha-Tropinyl Benzilate	Diethyaminoethyl Benzilate
Mouse	I.V.	22	14	29	14
	I.P.	110	--	--	115
	S.C.	215	--	--	350
	P.O.	490	--	55	160
Rat	I.P.	--	--	--	113
	P.O.	--	--	--	166
Guinea Pig	I.V.	14	--	--	--
	I.P.	--	--	--	115
Rabbit	I.V.	10	--	--	15
	I.P.	--	--	--	115
Cat	I.V.	12	--	--	--
Dog	I.V.	12	--	--	--
Pig	I.V.	5	--	--	--
Goat	I.V.	7	--	--	--

TABLE I-3

Single-Dose LD_{50}s of Phenylisopropylglycolic Acid Esters

Animal	Route of Administration	LD_{50}s, mg/kg EA 3834	LD_{50}s, mg/kg 302,668
Mouse	I.V.	63.0	81.1
	I.P.	--	120.0
	P.O.	--	383.0
Rat	I.V.	--	63.5
	I.P.	--	153.4
	I.M.	262	--
Rabbit	I.V.	25.1	30.0
Cat	I.V.	--	49.0
Dog	I.V.	49.5	65.0
Monkey	I.V.	29.4	100.0

TABLE I-4

Single-Dose LD_{50}s of Nonester Anticholinergic Compounds

Animal	Route of Administration	LD_{50}s, mg/kg Mepiperphenidol	 Benzetimide
Mouse	I.V.	20.0	46.0
	S.C.	--	640
	P.O.	970.0	305
Rat	I.V.	--	37.6
	S.C.	--	160
	P.O.	--	404
Guinea Pig	S.C.	--	153
	P.O.	--	825

TABLE I-5

Single-Dose Minimal Effective and Lethal Intramuscular Doses of TAB

Animal	ED_{50}, mg/kg[a]	LD_{50}, mg/kg[a]
Male mice	--	61 ± 4.2
Male rats	36 (27, 48)	251 (210, 301)
Female rats	36 (10, 134)	154 (131, 179)
Male rabbits	45	66
Dogs	21	80
Monkeys	21	50

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[a] Figures in parentheses are 95% confidence limits. The standard deviation of the mean is given for mice. Single values were derived from experiments whose results were not suitable for calculation of appropriate statistics.

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APPENDIX J

ANTICHOLINERGIC DRUGS AND THE EEG

by

Max Fink, M.D.

This review examines the effects of anticholinergic compounds on the human central nervous system, in relation to their military testing. Such compounds were tested in volunteers to define their role as incapacitating agents for military use. The question presented is whether, as tested, the chemicals are likely to produce adverse health effects or delayed sequelae in the test subjects, or whether it is possible even to predict that likelihood This review focuses on the data derived from electroencephalography.

The data include protocols of military experiments declassified for this review, reports published in the scientific literature on animals and man, and my own studies. Between 1956 and 1966, experiments were undertaken in my laboratories in New York and St. Louis to define the effects of anticholinergic drugs on brain function and behavior. We sought to determine whether any of the substances had persistent behavioral effects that could be useful in treating the severely mentally ill (1-7).

Atropine and scopolamine (hyoscine) are prototypic anticholinergic compounds. Their effects are principally antimuscarinic. Compounds usually classified with these prototypes are those used in the treatment of parkinsonism--such as procyclidine (Kemadrin), benztropine mesylate (Cogentin), and trihexyphenidyl (Artane)--and such medicinals as glycopyrrolate (Robinol) and methantheline (Banthine). Such experimental anticholinergic compounds as Ditran, JB-329, JB-336, and WIN 2299 are included in the same class. In some studies, particularly EEG studies in patients, the tricyclic antidepressants imipramine and amitriptyline have been shown to have atropine-like properties.

Electroencephalography developed from studies published in 1929. In the early studies, changes were assessed by visual inspection of ink-written records. These have since been replaced by electronic and, lately, digital computer methods of quantitative analysis. These methods provide excellent assessments of minimal changes in brain function in man. Unfortunately, the military reports are limited to inspection.

LITERATURE REVIEW

The effects of anticholinergic drugs on brain function have been studied extensively. CNS effects in man are defined principally on the basis of self-reports, observer evaluations,

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neuropsychologic tests, and physiologic measures. Of these, the EEG, especially in its present quantitative form, is a sensitive index of changes in CNS activity, with particular relevance to human performance and states of vigilance (8,9).

Anticholinergic drugs have well defined effects on the EEG, accompanied by measurable behavioral effects. In most drug studies, EEG patterns correlate well with behavioral changes. Studies of atropine in animals, however, elicited reports that their EEG showed increased amplitudes and lowering of frequencies at times when the animals were apparently restless. Animals examined in halters often exhibited running movements, but their EEG patterns were similar to those seen in deep sleep (10). The apparent purposeful movements associated with "sleep-like" EEG records led some observers to define an "EEG-behavioral dissociation" with anticholinergic drugs (1, 11-15). In studies of psychoactive substances in man, we and others found a close relationship between the changes in EEG patterns and the behavioral effects of drug administration (8). The difference in observations between animals and man led to a symposium in 1966 that summarized the data available to that time (1). The symposium participants concluded that the apparent "dissociation" in EEG and behavior with anticholinergic drugs was limited to observations in animals and was an artefact of the gross nature of the measures used in animal trials, rarely, the inability to assess changes in cognition, vigilance, mood, and affect (which are principal targets of anticholinergic drugs).

Anticholinergic drugs have a characteristic dose-related effect on brain function, and particularly on the resting, alert scalp-recorded EEG (3,4,6,7,16-27).

Low doses, such as 1-2 mg of atropine, are sufficient to induce mild tension, irritability, and anxiety. The subjects are aware of changes in their perception and mood, and they make errors on cognitive tests. At these times, the EEG patterns exhibit an increase in high frequencies and decreases in the mean alpha frequency and in the amplitude of the dominant (alpha) frequencies. These effects may be accompanied by a decrease in heart rate and some minimal effects on salivation and skin conductance (28, 29). With higher doses, such as 10-30 mg, or repeated administrations, the subjects become delirious, showing restlessness, impairment of motor and sensory functions, cognitive defects, illusory sensations, and thought disorder, including hallucinations and delusions. Heart rate is increased, and the peripheral effects of dry mouth and skin, decreased urination, and difficulty in near vision are prominent. The EEG shows an increase in slow waves, a decrease in mean frequency, a decrease in the percent time and amplitudes of alpha activity, and an increase in the high frequencies, which can be seen to be "riding" on the slow waves. There is a direct association between the amount of EEG fast waves with behavioral restlessness and the amount of EEG slow waves with stupor and cognitive defects. At "toxic" doses, patients are in stupor or coma, with rapid heart rate and lowered blood pressure. The EEG demonstrates persistent high-voltage slow waves, with a minimum of alpha and high frequencies.

In epileptic patients, single intramuscular administrations of atropine at up to 2 mg/kg (30) and 1.2 mg of intravenous hyoscine (24) elicited epileptic spike EEG activity. The changes were transient, and the records and behavior of the patients returned to normal within 24 h.

EEG and behavioral effects may be modified by the concurrent administration of other drugs. Thus, atropine (or Ditran) and chlorpromazine resulted in behavioral stupor that was very deep (allowing surgery without pain responses), accompanied by persistent EEG high-voltage slow waves and decreases in fast waves. When Ditran and yohimbine or Ditran and imipramine were given together, restlessness increased, as did the ratio of fast waves to slow waves in the EEG. When patients who exhibited a toxic delirium to Ditran or atropine were given tetrahydroaminacrin (THA), the stupor was relieved and the EEG showed decreases in both the low and high frequencies (1,7,31).

After acute administration of various compounds, the time for recovery varies with dose--at low doses, the peak effects on parenteral administration are seen in 0.5 h and last for up to 6 h; at high doses, the effects persist for up to 24 h; at toxic doses, there is a return to baseline values the second day after administration. Followup EEG data are limited, the principal data being reports of atropine toxicity. The few statements about EEGs sugggest that the effects are gone within a few days of the last exposure (32-37).

In studies of imipramine, some patients developed an acute psychotic reaction. These patients were identified as being young and as exhibiting a "schizophrenic" syndrome--an observation that led to administration of imipramine as a test of "schizophrenia" (2,38).

Although no long-term EEG study of anticholinergic drugs is available, one study of cholinesterase inhibitors may be cited. Duffy et al. (39) reported persistent quantitative differences in EEG patterns between workers exposed to the organophosphate compound sarin and a control group of workers in the same plant not so exposed.

MILITARY DATA

The principal military data on EEG studies are in the summaries provided in Case Report Summaries - Anticholinergics, and an addendum provided in a letter by Dr. Frank Marzulli of October 8, 1981. This latter summary included all the useful EEG records cited in the formal protocols of the military studies. No EEG records were found of subjects receiving atropine, scopolamine, EA 3443, or EA 3167. Of the available records, five were related to BZ, two to EA 3580, and one to EA 3834.

The reports stated that the pretreatment records were within normal limits and the postexposure records, taken at various times, were also within normal limits. The records were assessed visually. The reports did not state the conditions of testing, nor is it clear how long after exposure the testing was done.

These records were customary for the time and reflected nonspecific effects that are similar to those reported for many CNS active compounds. The reports did provide neuropsychologic-test data, however, which indicate that the effects of exposures to the anticholinergic substances on the military volunteers were transient, reverting to baseline values within a few hours or, in a few tests repeated at longer intervals, within a few weeks (40-49. In other studies of the relationship between the changes induced in EEG and in neuropsychologic tests by CNS-active drugs, there was a parallel in the onset and duration of the effects in the different measures. Arguing by analogy, we would anticipate similar reversions to normal for the EEG changes in these volunteers.

Conclusions

In the experiments in which patients and normal volunteers were exposed to single or multiple doses of anticholinergic drugs, a consistent pattern of EEG and behavioral change has been described. In most volunteer studies, doses have been low, exposures usually single, and effects transient. There is no evidence of persistence of behavioral or EEG effects in these experimental trials (50).

In patients who have been given heroic doses of atropine and scopolamine (up to 250 mg) and in whom the doses have often been repeated three times a week for up to 4 mo, there have been few signs of persistent toxicity. The patients have been subjected to periods of coma lasting up to a day. Death has been reported in only one instance. In many patients, the persistent effects have been considered salutary--i.e., the patients have been considered improved in mental state and discharged to the community! In the remainder, they have been reported to be no worse than before treatment. Considering the many hundreds of patients so treated and the continuation of this form of therapy in patients in eastern Europe today, it is unlikely that there is a behavioral syndrome of toxicity.

Focusing exclusively on the anticholinergic properties of the drugs examined in military volunteers, considering the low doses used and the minimal exposures, and aware that heroic doses of anticholinergic drugs (inexplicably) fail to stimulate a defined toxic syndrome, we deduce that the single exposures of doses of anticholinergic drugs used in the volunteers were insufficient to stimulate a persistent toxic syndrome. The data available are sufficient to conclude that, as tested, the chemicals are not likely to produce adverse health effects.

For a more definitive conclusion, a prospective study or a study similar to that reported by Duffy *et al*. (39) in parkinsonism patients or industrial workers exposed to anticholinergics is required.

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APPENDIX K

BIOCHEMICAL ASPECTS OF ANTICHOLINERGIC CHEMICALS

by

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There have been few published studies--an example is that by Jovic and Zupanc (1)--on the biochemical mechanisms associated with quinuclidinyl benzilate (BZ). A major attribute of atropine, scopolamine, and other anticholinergic compounds is their ability to interact with postsynaptic muscarinic binding sites. It is through such an action on smooth muscle that our ideas about BZ and other potent anticholinergics have developed. A property common to all is a high affinity for muscarinic receptor sites ($K_D = 0.05 - 0.1 \times 10^{-9}$ M). A regional study with [^{3}H]BZ of binding capacity in hippocampus, frontal cortex, and caudate nucleus of human brain showed (2), small decreases in these areas in Huntington's chorea patients as compared with normal human brain. Atropine and BZ were alike in binding capacity to these sites. Because of the high affinity of BZ for muscarinic receptors, Yamamura and Snyder (3) were first to show its binding to postsynaptic sites, but failed to find evidence of presynaptic sites in the CNS. The suggestion by Polak and Meews (4) that the increased release of acetylcholine in vivo could be accounted for by blockade of "presynaptic" muscarinic receptors therefore requires another explanation.

An early finding by Sacktor (5) is that acetylcholine (8.6×10^{-5} M) increases phosphatidyl-L-serine, as well as phosphoinositide and phosphatidic acid. In addition, atropine (1.6×10^{-6} M) and BZ (7×10^{-5} M) blocked the ACh-stimulated incorporation of $^{32}PO_4$ into phosphoinositides, but stimulated incorporation of labeled phosphate into phosphatidyl serine. This suggests that, although events related to muscarinic receptor action are blocked by antimuscarinic drugs, other actions may be stimulated. It is known that calcium ions activate phospholipase-C and stimulate the turnover of phospholipids, such as triphosphoinositides, in excitable membranes. Calcium ions have diverse functions in the regulation of cell function. They are required for the release of acetylcholine (6) in neuromuscular transmission and they influence threshold and other attributes of CNS action potentials. It is estimated that intracellular "ionized" calcium is low in neurons (about 10^{-7} M). A small rise in intracellular calcium will produce a sharp rise in K^+ conductance, hyperpolarization, and depression of excitability. The slowed excitation of central neurons by the muscarinic actions of acetylcholine may be countered by anticholinergic drugs and thus account for many of the central effects of BZ in volunteers reported by Ketchum (7) and by Sidell (8). Most prominent among these are amnesia, confusion, and disorientation lasting 2 to 6 d. Although the effects of BZ are qualitatively similar to the pharmacologic actions of atropine and scopolamine, the potency and especially the duration of action of BZ are considerably greater. That situation is very likely related to properties other than the binding affinity, which is about the same for all these compounds for the muscarinic receptor ($K_D = 10^{-9}$ M).

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The π -bond properties of quinuclidinyl compounds probably account for their "sticking" to tissue constituents for long periods. Experimentally, a 5-mg/kg dose of radiolabeled BZ prevented auditory stimuli for 7-8 d, but labeled drug was hardly detectable in brain after 3 d. Unfortunately, the only thorough study was that by Gosselin et al., (9), on [^{14}C]atropine, so direct comparisons are difficult. Although atropine abuse with 2,5-dimethoxy-4-ethyl amphetamine (STP) has been reported (10), the number of "bad trips" made its popularity short-lived. The absence of further reports on abuse of atropine-like drugs suggests that their abuse by the volunteers in question does not constitute a long-term problem.

Administration of atropine or scopolamine to animals increases acetylcholine output from the exposed cerebral cortex (11). This has led to the hypothesis of a direct effect on presynaptic muscarinic receptors that regulate ACh release. The finding of release when Ca^{2+}-free Ringer's solution is present (12) led to the suggestion that interneurons are more sensitive to a local decrease in Ca^{2+} ions than the cholinergic nerve endings whose release they modulate. Alternatively, the ability of BZ, Ditran, and other anticholinergic drugs to cause the release of calcium from intraneuronal binding sites (13) could also explain the in vivo observations. The role of ionized calcium in the release of ACh in neuromuscular and electroplax preparations is well characterized. It is persuasively argued that, when ACh emerges from a terminal, it has to pass through some sort of "gate" that is a Ca^{2+}-dependent channel; the "gate" is more likely to be open when the terminal is depolarized. The suggestion that control of ACh release is at the level of calcium displacement by BZ is important, because it is the Ca^{2+}-dependent property of "anticholinergic" drugs, and not their antimuscarinic actions, that explains why BZ and Ditran are much more active than atropine centrally. They appear to be equally effective peripherally, when comparisons are based on affinity for muscarinic binding sites.

Calcium ion contents can be regulated by any of the membrane systems that are in contact with the cytosol, e.g., plasma membrane, endoplasmic reticulum, and mitochondrial inner membrane. In brain, mitochondria are in the highest concentration at nerve terminals and represent the major supplier of cellular energy (ATP). Mitochondria can take up Ca^{2+} ions by an energy-requiring process and release free Ca^{2+} in exchange for Na^+ ions. The high capacity of isolated brain mitochondria to transport Ca^{2+} ions has led to the suggestion of a significant role for this organelle in ionic homeostasis. The early observations on energy metabolism demonstrated that Ditran and BZ selectively interfered with the increased energy needs in vitro of depolarized cerebral tissue. In contrast, atropine and scopolamine were either without effect or active only at much higher concentration. In the presence of isolated brain mitochondria, Ditran (JB339) was shown to interfere with ATP production (13). Calcium retention by brain mitochondria is lost when energy generation (ATP) is interfered with (14). The significance of these and other studies is that BZ and Ditran are quantitatively different from the less-potent atropine and scopolamine. A selective action on Ca^{2+} channels by BZ and Ditran on particularly vulnerable neurons would explain the unevenness in the correlation between the receptor-binding properties and behavioral alteration by antimuscarinic compounds in animals and man.

CONCLUSION

Reports of biochemical effects of anticholinergic compounds contain data on animals and may or may not permit extrapolation to man. There is evidence that the actions of quinuclidine derivatives are longer-lasting and the limited metabolic data available suggest that they may be retained in tissues for a longer period. Peripherally, the benzilates are acetylcholine antagonists with high affinity (low KDs). They bind reversibly to muscarinic receptors. Centrally, however, their actions are more complex, and pharmacodynamic and pharmacokinetic properties play an essential role. The available evidence is not entirely convincing that their basis of central action is through postsynaptic muscarinic receptor binding, and their presynaptic role in calcium metabolism must be seriously considered. Without morbidity data, our present biochemical information cannot help to predict long-term effects of exposure to anticholinergic agents.

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APPENDIX L

STRUCTURE-ACTIVITY RELATIONS OF THE CENTRALLY ACTIVE ANTICHOLINERGIC AGENTS

by

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The structure-activity relationships of a large number and variety of anticholinergics have been described in several reviews (1-3), and only the general features will be described here. The main difficulty in comparing chemical structure with psychopharmacologic potency is that unequivocal behavioral measurements of potency are deficient. Although some anticholinergics have been evaluated for their psychotomimetic potency in man (2,4), the comparisons are based largely on a battery of psychopharmacologic measures in animals, including hyperactivity (5), swim maze (6), characteristic exploratory head movements (2), and operant conditioning (7-9). The limitations and reliability of these comparisons have been discussed elsewhere (3), and they do permit generalizations concerning the relative ability of the anticholinergics to produce behavioral disturbances in animals that may reflect psychotogenic potency in man. Structural variations in the heterocyclic amino alcohol result in marked changes in potency; the most potent anticholinergic is 3-quinuclidinyl benzilate, with its rigid conformation (Figure L-1).

With respect to the acid moiety of the anticholinergics, the following structure-activity relationships are found:

As R_1 is increased from methyl to higher alkyls or becomes hydrogen, alkenyl, amino, or aminoalkyl, psychotropic potency diminishes without much effect on peripheral anticholinergic action.

R_2 should be an unsubstituted phenyl group, wherase R_3 must be either a cycloalkyl, alkynyl, thienyl, or unsubstituted phenyl. Alkyl, aryl, halide, or hydroxyl substituents on the phenyl rings abolish central action and diminish anticholinergic potency. R_2 and R_3 can also be replaced by hexahydrofluorenyl (10).

o As "n" is increased beyond 2 or "Y" beyond zero, psychotropic, but not anticholinergic, potency decreases. The position of the ester side chain affects central, but not peripheral action, with the 4-piperidyl ester being most potent, the 3-ester second most and the 2-ester least.

o R_4 must be a hydroxyl group whereas compounds with hydrogen or an isosteric methyl group are devoid of central action and have diminished anticholinergic action. If R_4 is hydrogen and the hydroxyl group is present on phenyl, central potency is retained.

The duration of the psychotropic action of the various anticholinergics depends both on the type of heterocyclic amino group and on R_2 and R_3. The quinuclidinyl and pyrrolidyl amino derivatives tend to be longer-acting than piperidyl, tropanyl, or granatonyl; a cycloalkyl group in R_2 or R_3 prolongs duration. An alkynyl group in R_2 or R_3 decreases duration, whereas increasing chain length or branching of the alkynyl group correspondingly increases duration.

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STEREOSPECIFICITY

The anticholinergic glycolate esters of heterocyclic amino alcohols, including scopolamine and related natural alkaloids, exist as optical isomers, resulting from the asymmetric C of both the amino alcohol and acid moiety. Of the two enantiomers of 3-diphenylacetyl quinuclidine, the (-) isomer had 25 times the antispasmodic potency of the (+) isomer; however, the isomers were of equal toxicity, whereas no difference in antispasmodic potency was noted between the two isomers of the quaternary derivative of 3-quinuclidinyl benzilate (11). With respect to their central action, the (+) and (-) enantiomers, which were prepared from the respective quinuclidinols resolved with (+)-camphor-10-sulfonic acid (11), differ markedly in potency (12,13). The (-) isomer was reported to have about 20 times the potency of the (+) isomer in producing ataxia in dogs (12); however, with the use of more elaborate behavioral measurements in cats, the potency difference was in excess of 100-fold (13). It is conceivable that the (+) isomer of 3-quinuclidinyl benzilate is devoid of activity on the central nervous system, and the slight activity observed may be attributed to a 1% contamination by the (-) isomer.

COMPARISON OF ANIMAL AND HUMAN DATA WITH VARIOUS ANTICHOLINERGIC DRUGS

In general, there is good agreement between the relative pharmacologic potency of various anticholinergic psychotomimetic agents in animals and that in humans. The comparison is applicable to both central and peripheral effects of the anticholinergics and to their duration of action. Table L-1 summarizes the data obtained on the Edgewood volunteers who were given a single dose of an anticholinergic agent. Central nervous system (CNS) potency and peripheral antimuscarinic potency are expressed on an arbitrary scale of 0-10, with 10 being the most potent. Most of the agents used were of comparable peripheral antimuscarinic potency, whereas the CNS potency extended over the whole range.

The CNS data in Table L-1 can be summarized as follows. BZ and other quinuclidinyl glycolates containing at least one phenyl and a cycloalkyl group in the acid moiety were the most potent and had the greatest duration of action. The corresponding piperidyl glycolates were one-fifth to one-half as active as the quinuclidinyl esters. It can be concluded from such structure-activity studies, which are based on extensive psychopharmacologic stests in both animals and human volunteers, that BZ is the most representative of the most potent anticholinergics. However, atropine and methylatropine are the most representative of the relatively inactive anticholinergics and could serve as control drugs, particularly because atropine is equipotent, although of shorter duration, to BZ in peripheral anticholinergic effects.

SITES OF ANTICHOLINERGIC ACTION IN BRAIN

Studies have been performed on the regional and cytoplasmic distribution of high-affinity radiolabeled anticholinergics in mammalian brain (14,15). In monkey brain, the regional distribution of [^{3}H]3-quinuclidinyl benzilate was found to correlate well with other cholinergic measures, such as [^{3}H]choline uptake, choline acetyltransferase, and acetylcholinesterase (16). By far the highest level of all four parameters was in the putamen and caudate nucleus. The cerebral hemispheres, amygdala, and hippocampus contained about half the concentration of the benzilate, but the other concentrations were small fractions of those in the caudate nucleus. Apart from the caudate nucleus, the correlation between the distribution of the anticholinergics and the measures of cholinergic function was not impressive. By determining the extent to which [^{3}H]3-quinuclidinyl benzilate binding was diminished by pretreatment of rats with atropine, the degree of specific binding of the anticholinergic can be determined in various brain areas (16). It appears that, although a correlation was found in some brain areas (e.g., the caudate nucleus,) between the distribution of the anticholinergics and other measures of cholinergic action, the binding studies do not permit the conclusion that the drugs' distribution is an accurate reflection of the pattern of muscarinic cholinergic receptors in brain.

The binding of [^{3}H]-anticholinergics to tissue preparations after lesions are produced in specific brain areas is another means of measuring specific receptors for the anticholinergics. If nerve terminals of the cholinergic afferents to the hippocampus contain muscarinic cholinergic receptors, as suggested by pharmacologic studies (17,18), then lesions in the septal-hippocampal cholinergic tract should reduce the drugs' binding--a conclusion that was experimentally verified (19).

COMPETITION BETWEEN BEHAVIORAL POTENCY AND RECEPTOR AFFINITY

To determine whether the centrally active anticholinergics bind to a physiologic receptor, an attempt was made to correlate inhibition binding constants of the various anticholinergic agents with their psychopharmacologic potency (20). Such a correlation between behavioral and binding data has been attempted with a series of 14 glycolate esters (Figure 2). A linear relation was observed between the behavioral data and the logarithm of the inhibition constants for binding. The behavioral data were taken from previously published accounts and are expressed quantitatively as BDI (behavioral disturbance index, or BDI (21). From the evidence presented in this study, it can be concluded that [^{3}H]quinuclidinyl benzilate binds to a muscarinic site in brain that is involved in producing the behavioral disturbances elicited by the glycolate esters. This conclusion is based on the low K_d, the saturability and stereospecificity of binding competition studies, and especially the reasonable correlation observed between behavioral and binding data (Figure 2). Although the correlation can be useful in predicting the behavioral potency of new glycolate esters, on the basis of their inhibition constants, there are notable exceptions, such as atropine, scopolamine, and compound IV. All three drugs had very high affinities for the [^{3}H]quinuclidinyl benzilate binding site, but their behavioral potencies were relativey low. They all contain heterocyclic ring systems other than piperidine or quinuclidine. In a previous study, in an attempt to correlate the behavioral potencies of a series of glycolates with some physical constants, the correlation tended to be excellent for the quinuclidinyl and piperidyl esters, but not for those having other heterocyclic amino rings, such as tropanol and granatonol. However, an excellent correlation was observed between affinity constants and the ability of the anticholinergics to block the acetylcholine-induced contraction of ileum, the correlation being independent of the type of heterocyclic amino ring (20).

A comparison of the receptor binding affinities of the two optical isomers of 3-quinuclidinyl benzilate reveals that the (-) isomer has about 50 times the affinity of the (+) isomer for a synaptic-membrane preparation from rat caudate nucleus (unpublished). It had been reported that the (-) isomer had only 20 times the affinity of the (+) isomer for a neural-membrane preparation from whole rat brain (20). The reasons for the difference may be that caudate nucleus contains a greater concentration of cholinergic receptors and that a purified synaptic membrane preparation was used in the later study. The relative binding affinities of the two isomers however, still had less than the 200:1 potency ratio found in the cat behavioral test (22). A plausible explanation is that the specific neurons associated with the behavioral disturbances of the drugs may have a greater degree of binding stereospecificity than that exhibited by membrane preparations from either whole brain or caudate nucleus.

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TABLE 1

Structure-Activity Data on Anticholinergics in Human Volunteers

Code Name	Dose µg/kg	Duration of Effect, h	Potency[a] Peripheral	CNS	No. Subjects
BZ	5-8	48-96	10	10	354
22,608	1-2	48-72	10	10	21
EA 3167	3-4	48-120	10	10	24
301,060	3-5	48-120	10	9	29
CS 27349	7-8	6-24	10	5	50
302,282	7-14	6-12	10	4	8
EA 3580	4-37	12-48	8	3	136
EA 3443	4-60	24-48	8	3	101
302,196	22-54	4-10	8	4	56
Scopolamine	4-16	6-24	10	5	637
302,537	4-6	6-24	10	4	18
Ditran	100	12-24	10	3	12
EA 3834	3-24	12-24	9	4	171
302,668	13-18	12-48	8	8	39
Atropine	1-70	12-24	10	1	602
Benactyzine	100	6-24	4	1	17
Methylscopolamine	1-30	6-30	5	0	66
Methyl atropine	2-20	6-48	8	0	15
302,368	3-4	6-24	10	1	10
Atropine+benactyzine	(100+20)	4-10	10	1	5

---

[a] Potency is expressed in terms of peripheral (mydriasis, dryness of mouth, etc.) and central nervous system (CNS) effects. The latter involve confusion, hallucinations, memory loss, and delirium. Drugs are evaluated relative to BZ.

Figure F-1 Chemical structure of various heterocyclic amino esters of benzilic acid. I = 3-quinuclidinyl, II = *N*-methyl-4-piperidyl, III = *N*-methyl-3-piperidyl, IV = *N*-methyl-3-pyrrolidyl, V = *N*-methyl-*N*-methyl-2,3-piperidienyl, VI = 1,2,2,6-tetramethylpiperidyl, VII = *N*-methyl-3-tropanyl, VIII = *N*-methyl-3-granatonyl, IX = 1-pyrrolizidinyl, X = 1,2,2,6,6-pentamethyl-3-piperidyl. R = benzilate. Psychotomimetic potency decreases from I to X.

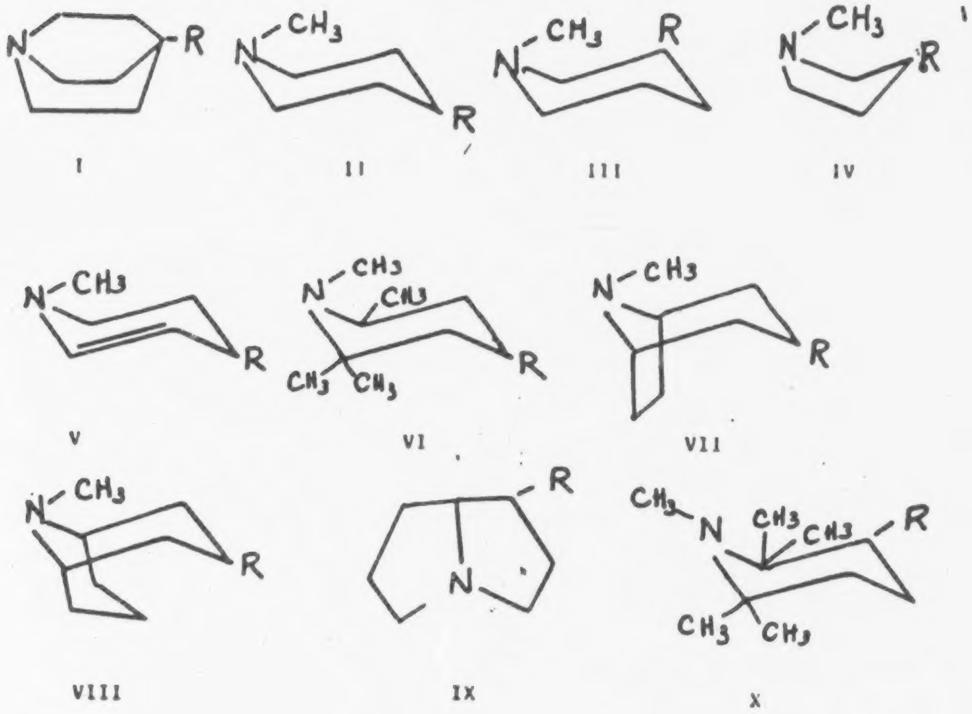
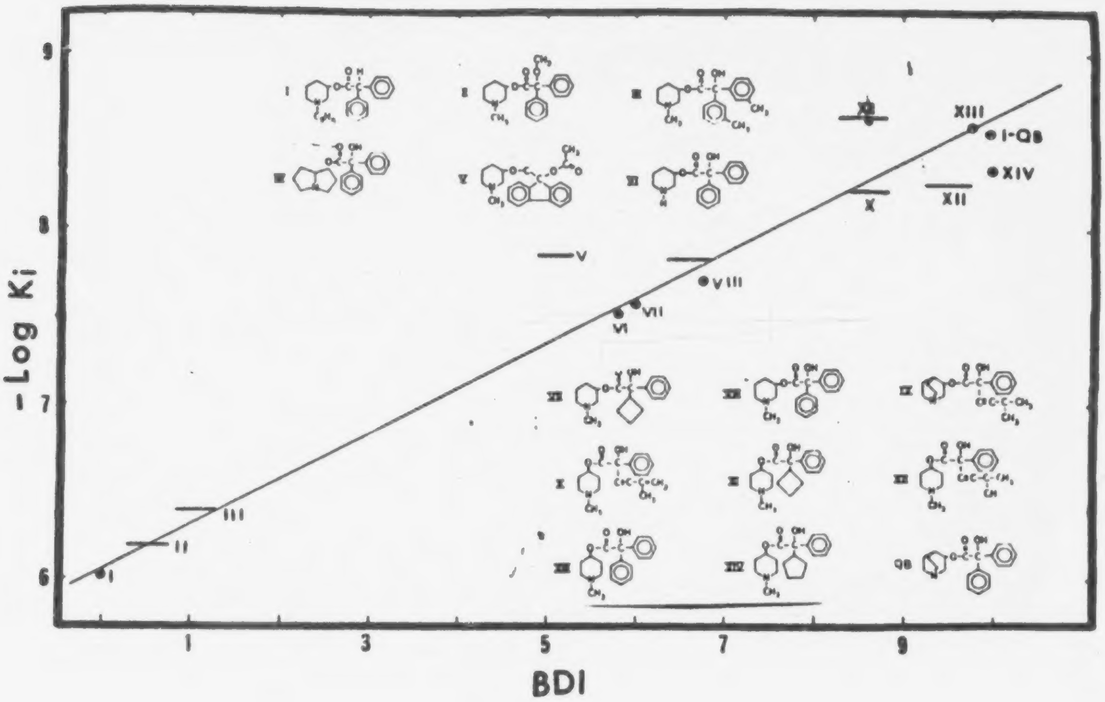

N
R
I
N
CH3
R
II
N
CH3
R
III
N
CH3
R
IV
N
CH3
R
V
N
CH3
CH3
CH3
CH3
R
VI
N
CH3
R
VII
N
CH3
R
VIII
R
N
IX
CH3
N
CH3
CH3
R
CH3
CH3
X

Figure F-2 Correlation of binding affinity of various centrally active anticholinergics to their behavioral disturbance index (BDI). Binding affinity is measured by K_i, the drug concentration producing 50% inhibition of $[^3H]$ quinuclidinyl benzilate binding to brain-membrane preparations. BDI = composite index of behavioral measurements in various animals as a measure of psychotropic potency. Data adapted from Baumgold *et al.*(20).



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Possible Long-Term Health Effects of Short-Term Exposure to Chemical Agents

Volume 2

Cholinesterase Reactivators, Psychochemicals, and Irritants and Vesicants

POSSIBLE LONG-TERM HEALTH EFFECTS OF SHORT-TERM EXPOSURE TO CHEMICAL AGENTS

Volume 2

Cholinesterase Reactivators, Psychochemicals, and Irritants and Vesicants

Prepared by the

Panel on Cholinesterase Reactivator Chemicals
Panel on Psychochemicals
Panel on Irritants and Vesicants

Committee on Toxicology
Board on Toxicology and Environmental Health Hazards
Commission on Life Sciences
National Research Council

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PREFACE

The Department of the Army asked the Committee on Toxicology, in the Board on Toxicology and Environmental Health Hazards of the Commission on Life Sciences, National Research Council (NRC) to conduct a study of the possible chronic adverse health effects on servicemen of experimental exposure to various chemicals at the U.S. Army Laboratories (formerly the Army Chemical Center), Edgewood, Md. The Edgewood tests were conducted over a 20-yr period ending in 1975, to learn how five major classes of chemicals tested for various military applications may affect humans. Some 6,720 soldiers took part in the programs, and 254 chemicals were administered by various routes.

In 1982, the Committee reported (Volume 1) on possible long-term effects of two pharmacologic classes of chemicals (anticholinesterases and anticholinergics) tested at Edgewood. This volume reports on long-term effects of three additional classes of chemicals tested at Edgewood: cholinesterase reactivators, psychochemicals, and irritants and vesicants. Studies of these substances, including LSD (discussed in a separately prepared report), constituted the main thrust of the Edgewood experiments.

After completion of Volume 1, three new panels were established to identify and assess evidence on the possible long-term health effects or delayed sequelae of the three chemical classes tested. This was done over a period of a year, during which each panel met three times. Pertinent material was examined to evaluate the possibility that experimental exposure of soldiers may have resulted in untoward health effects. The three panels were separately concerned with four cholinesterase reactivator chemicals (oximes); two types of psychochemicals (phencyclidine and dimethylheptylpyran and congeners), administered in pure form, as opposed to street drugs; and mustard gas and several lacrimatory and respiratory irritants (such as CN, CS, CR, and CA).

The charges to each panel were as follows:

- To determine whether there was sufficient evidence to assess the likelihood that the test chemicals had had long-term health effects or delayed sequelae.

- To determine, on the basis of this evidence, whether the chemicals, as administered, are likely to have produced such adverse effects in the test subjects.

As part of this undertaking, the NRC staff conducted interviews with administrators, investigators, nurses, and technicians and reviewed documents to identify practices and procedures followed in testing chemicals at Edgewood. The interviews included persons who had a wide spectrum of viewpoints, but who were essentially in agreement regarding the conduct of human testing. Committees were formed at Edgewood to review classified chemicals and develop reports for declassification and use by NRC panels. Extensive extracts were prepared of preclinical animal and human protocols and of technical reports at Edgewood libraries and other facilities. A repository was established at Edgewood (August 1980) for storing selected reports and needed information obtained from other sources. Research and experimental case files on volunteers were extracted and summarized. Digests of the literature were prepared.

This report presents the conclusions of the three panels based on available toxicologic and epidemiologic data, including specific information on the frequency, routes, and amounts of the substances administered and the known immediate and long-term effects of the substances under the prevailing experimental conditions.

A third report is intended to provide a final evaluation of the chemicals tested. Volume 3 will report on the current health status of the soldier participants on the basis of a recent questionnaire and interpret its impact on the conclusions reported in Volumes 1 and 2. The followup questionnaire to volunteer subjects may add descriptive data, but, because of the limited sample size, it is unlikely to provide evidence of a cause-and-effect relation between exposure to these chemicals and development of delayed disease.

EXECUTIVE SUMMARY

In 1980, the Board on Toxicology and Environmental Health Hazards of the National Research Council's Commission on Life Sciences began a program to evaluate the long-term health effects of chemical agents administered to military volunteers at the Army Chemical Center, Edgewood, Md., during the 1950s, 1960s, and 1970s. This work was conducted at the request of the Department of the Army. The tests were conducted to find out how various potential chemical warfare agents affect human performance. It was felt that animal tests were inadequate for this type of evaluation and that only humans could provide definitive information.

The first report (Volume 1) reviewed data on 15 anticholinesterase and 24 anticholinergic agents, to which almost half of some 6,700 subjects were exposed at Edgewood.

The current report, prepared by three panels of the Board's Committee on Toxicology, evaluates possible delayed health effects of three additional classes of compounds that were tested on most of the remaining volunteer subjects: cholinesterase reactivators, psychochemicals, and irritants and vesicants (blistering chemicals). The cholinesterase reactivators, of which there are four, are used as antidotes for anticholinesterase poisoning. The psychochemicals include phencyclidine, an anesthetic with substantial disorienting effects that is also available (with impurities) as the street drug PCP, and 10 related dibenzopyrans that are central nervous system depressants capable of producing orthostatic hypotension. The irritants include the well-known lacrimatory chemical CN, the riot-control agent CS, and other ocular and respiratory irritants. Mustard gas was the vesicant whose effects were studied.

The Committee established three panels to identify and assess evidence on the possible long-term health effects or delayed sequelae of the chemicals tested. As in the work that led to Volume 1, the chairman of each panel was selected from the Committee on Toxicology.

The specific charges to each panel were as follows:

- To determine whether there was sufficient evidence to assess the likelihood that the test chemicals had had long-term health effects or delayed sequelae.

- To determine on the basis of this evidence whether the chemicals, as administered, are likely to have produced such adverse effects in the test subjects.

The conclusions in this volume are based on available epidemiologic, toxicologic, and mortality data that were reported in Volume 1. They also rely on a review of test-subject exposure data obtained from Edgewood, all of which were available to panel members. Long-term clinical followup was not conducted. The subjects tested were healthier than the control subjects with whom they were compared, and both groups were healthier than the general population, reflecting the better health status of those in military service. The analyses presented here necessarily reflect the limitations of the available data. These conclusions might change in the light of information gained through a study of morbidity, which will be based on a questionnaire and an analysis of admissions to Army and Veterans' Administration hospitals (to be reported in Volume 3).

CHOLINESTERASE REACTIVATORS

On the basis of an examination of toxicologic literature, case reports from Edgewood volunteers, and a review of mortality data conducted by the National Research Council Medical Follow-up Agency, the Committee found no evidence of chronic disease in animals or humans associated with single or repeated doses of the cholinesterase reactivators (oximes). The lack of followup data on volunteers prevents certainty in predicting occurence or absence of delayed effects. The compounds are eliminated very rapidly from the body, but they produce a variety of acute effects that are short-lived and reversible, such as gastrointestinal distress after oral administration, pain at an injection site, dizziness, headache, and ocular discomfort. The Committee found no conclusive studies of carcinogenicity, mutagenicity, teratogenicity, or reproductive anomalies associated with the four oximes and therefore did not reach a conclusion in this regard.

PSYCHOCHEMICALS

The Committee found the evidence on the long-term health effects of the tested psychochemicals to be sparse. The target organs that may be involved in prolonged or delayed effects of phencyclidine are the brain and cardiovascular system. Target mental or cardiovascular effects did not take place within a week of exposure to the drug. No case reports have identified long-term effects or mental or cardiovascular effects soon after first exposure.

The margin of safety of a tested chemical is sometimes estimated by considering the ratio of the lethal dose to the pharmacologically effective dose (the dose at which some detectable biologic effect occurs). On the basis of animal data on the psychochemicals tested, the margin of safety for short-term effects is large for acute intravenous, intragastric, intraperitoneal, and subcutaneous administration and somewhat smaller for inhalation of the aerosolized form.

On the basis of the scientific literature alone, it is not possible to predict whether any long-term effects would be asociated with the small exposures used. However, evaluation of this toxicity literature and the Edgewood studies led this Committee to conclude that, at the doses and frequencies of phencyclidine used at Edgewood in a small number of test subjects, it is unlikely that detectable long-term or delayed effects have occurred or will occur.

Acute administration of the dibenzopyrans (dimethylheptylpyran and congeners) produced various degrees of physical incapacitation in Edgewood subjects, mainly because of moderate to marked and prolonged orthostatic hypotension. The duration and intensity of effects varied among most doses and subjects. Despite the variations, there is a large pharmacologic margin of safety in the use of these compounds in animals. The dibenzopyrans produced more potent long-lasting orthostatic hypotension and weaker (but otherwise similar) psychologic effects than Δ-9-tetrahydrocannabinol during the Edgewood experiments. There is no information on chronic effects of dibenzopyrans.

IRRITANTS AND VESICANTS

The Committee analyzed published studies describing the in vivo and in vitro properties of the agents used and reviewed short-term data collected by the U.S. Army on volunteers. The ability to provide definitive answers to the questions raised by the charge to the Committee was limited by the absence of long-term followup studies of the soldiers and by the sparseness of chronic effects studies of these compounds in animals or in humans after industrial exposure.

In general, the Committee found insufficient evidence to evaluate these chemicals, except mustard gas. Mustard gas is an experimental mutagen and human carcinogen at high doses.

Data on the irritants are insufficient to evaluate their mutagenicity, carcinogenicity, or other long-term effects. Tests of all theses chemicals involved few exposures and low doses.

MUSTARD GAS (H)

Mustard gas is highly reactive and has vesicant and systemic toxic effects. It is an alkylating agent that is mutagenic in various laboratory test systems, including mammalian germ cells, but data are inadequate to predict the extent of its genetic risk in humans. Mustard gas is also carcinogenic in experimental animals and humans. Other possible long-term effects of mustard gas are related to its local toxicity; specifically, it can cause blindness, possible skin tumors from some cases of permanent scarring of the skin, and chronic bronchitis. Reported instances of long-term injury, such as carcinogenesis in workers in a Japanese mustard production plant, were associated with exposure at high, long-term dosages. Information is insufficient to project risks associated with smaller exposures to mustard gas; however, serious long-term adverse effects in the small number of soldiers who received one or a few low-dose exposures at Edgewood seem unlikely (except for possible skin tumors and some cases of permanent scarring). Some of those exposed at Edgewood suffered skin injuries that took several weeks to resolve. However, in view of the small number of persons tested (about 150 healthy men) and the very low dosages involved, it is unlikely that a statistically significant increase in the risk of cancer or other chronic disease can be detected in those exposed to mustard gas at Edgewood. When exposed, the Edgewood subjects were wearing gas masks and impregnated clothing--an ensemble being tested for efficacy against toxic contamination.

o-CHLOROBENZYLIDENE MALONONITRILE (CS)

Results of experimental studies in microorganisms and short-term experiments in laboratory animals suggest that long-term medical abnormalities in soldiers exposed to CS are unlikely. Acute tissue changes produced in animals and humans seem reversible and not likely to become chronic in the absence of recurrent exposures. Followup information on the long-term state of health of exposed soldiers is not available, but no reports indicate that Edgewood subjects have experienced any long-term sequelae.

CHLOROACETOPHENONE (CN)

CN, a moderately toxic irritant, has immediate effects on the eyes, skin, and respiratory tract. CN is a strong skin-sensitizing agent, but is rarely lethal. The Committee found no evidence of lasting ocular or respiratory effects in 99 volunteers exposed experimentally at Edgewood between 1958 and 1972 when subjects were evaluated 2 wk after cutaneous administration or inhalation of aerosol. Allergic contact dermatitis or hypersensitivity in these volunteers on re-exposure to CN is possible. There has been no systematic study of the possible mutagenic and neoplasm-promoting effects of CN with current scientific methods.

DIBENZ[b,f][1,4]OXAZEPINE (CR)

CR, a mild lacrimatory irritant, manifests less acute toxicity than CN and CS. At low doses, it causes transient effects. There are a few studies on long-term health effects, including potential mutagenicity and teratogenicity. The available data are insufficient to predict long-term health effects. The small number of exposures and the small number of subjects exposed to CR at low doses at Edgewood make the occurrence of demonstrable effects in these subjects unlikely.

CHLOROPICRIN (PS)

PS is acutely toxic and has a variety of sensory effects in animals. It has not been evaluated thoroughly for mutagenicity or carcinogenicity. Like those exposed to mustard gas, the subjects exposed to PS were wearing gas masks, and small numbers of soldiers were exposed to small doses. PS is unlikely to have produced detectable long-term health effects in volunteers exposed at Edgewood.

BROMBENZYL CYANIDE (CA), DIPHENYLAMINOCHLORARSINE (DM), and 1-METHOXY-1,3,5-CYCLOHEPTATRIENE (CHT)

CA, DM, and CHT are unlikely to have produced measurable long-term health effects in volunteers exposed at Edgewood. But there are no specific toxicologic data on the mutagenicity and carcinogenicity of these compounds. CHT is less toxic than CN or DM when administered acutely.

NONANOYL MORPHOLIDE

The Committee does not expect long-term health effects in volunteers tested with nonanoyl morpholide at the dosages used at Edgewood. As with CA, DM, and CHT, specific toxicologic data regarding its potential in this regard are not available.

123 IRRITANT CHEMICALS

A total of 123 irritant chemicals were tested on only two subjects each. There are no data on their mutagenicity, carcinogenicity, or other long-term health effects. However, because the exposures were small, detectable adverse effects seem unlikely.

OVERVIEW

The Army's studies on human subjects were designed entirely to evaluate short-term physiologic and pharmacologic effects. A review of all available data reveals that these data are inadequate to provide definitive answers regarding the likelihood that the test chemicals produced or did not produce long-term health effects or delayed sequelae. Information on long-term health effects of the test chemicals on animals or humans is lacking, as is followup information on the current health status of the subjects. It therefore cannot be determined whether some subjects may have sustained long-term or delayed effects. Analysis of a questionnaire and of admissions to Army and Veterans' Administration hospitals (Volume 3) may provide further information on the current health status of these subjects.

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1

INTRODUCTION

In the spring of 1980, the Department of the Army asked the Committee on Toxicology of the National Research Council's Board on Toxicology and Environmental Health Hazards to study the possible chronic or delayed adverse health effects incurred by servicemen who had been exposed experimentally to various chemicals at Aberdeen Proving Ground, Edgewood, Maryland, during the years 1958-1975. The Edgewood tests were conducted to learn how potential chemical warfare agents might affect humans over a short period and how such affected humans might respond to therapy against such agents. The Army believed that relevant information could not be obtained from animal experimentation alone and that it was therefore necessary to confirm animal findings by using human volunteers.

Some 6,720 soldiers took part in this program. To understand the extent to which they might have experienced unanticipated long-term or delayed adverse effects, an extensive search of reports, records, and other data was undertaken. This search and study and evaluation of all available information on the involved chemicals themselves were accomplished by expert panels under the direction of the Committee on Toxicology.

To facilitate evaluation of the hazards associated with a long list of chemicals, the chemicals were grouped according to pharmacologic class. Of the five major groups of related chemicals, the largest and most important were the anticholinesterase and anticholinergic chemicals; these were reviewed first. Other chemical groups included cholinesterase reactivators, psychochemicals, and irritants and vesicants, and the effort culminated in a report issued in June 1982, Possible Long-Term Health Effects of Short-Term Exposure to Chemical Agents: Volume 1--Anticholinesterases and Anticholinergics.[1] That report dealt with 24 anticholinergic and 15 anticholinesterase chemicals that were administered to approximately 3,200 subjects.

The present report evaluates toxicologic and epidemiologic data relevant to the testing of approximately 750 subjects exposed to cholinesterase reactivators, about 260 exposed to psychochemicals, and 1,500 exposed to irritants or vesicants. A remaining group of subjects used largely in tests involving placebo or innocuous chemicals or conditions is available for comparison and will be discussed in Volume 3.

This report is the work of three panels of scientists--the Panel on Cholinesterase Reactivator Chemicals, the Panel on Psychochemicals, and the Panel on Irritants and Vesicants. The chairman of each panel was selected from the Committee on Toxicology, and the members were selected on the basis of their knowledge of the compounds in question or because they represented required disciplines.

Methods of selecting, testing, and handling subjects used for experimental work at Edgewood changed with time. Indeed, consent forms and methods of obtaining consent changed for all U.S. investigators during the period in which these tests were performed. A short history of the Edgewood testing program taken from Volume 1 is reproduced in Appendix A with a standard operating procedure for screening and selecting volunteers, which was issued by the Clinical Research Department at Edgewood on August 12, 1968.

LSD was among the psychochemicals tested at Edgewood, but its effects were not within the purview of the National Research Council's evaluations. Effects of LSD on 741 soldiers tested at Edgewood are described in a report by the U.S. Army Medical Department and the U.S. Army Health Services Command, issued in February 1980.[2]

Volume 3 will contain an evaluation of the current health status of Edgewood subjects based on their responses to a questionnaire and discussion and conclusions of the entire evaluation effort.

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2. U.S. Army Medical Department and U.S. Army Health Services Command. LSD Follow-Up Study Report. Project Director, David A. McFarling. 158 pp., February 1980.

2

CHOLINESTERASE REACTIVATORS

BACKGROUND

Rapid advances in chemistry during the nineteenth and twentieth centuries, coupled with the success of mustard gas as a toxic weapon in World War I, attracted attention to the warfare potential of chemical agents. This led to support for research on lethal nerve agents during and immediately after World War II. The research was followed by the development of treatment methods, and prominent among these was the use of cholinesterase reactivators to reverse the lethal effects of anticholinesterase nerve gases.

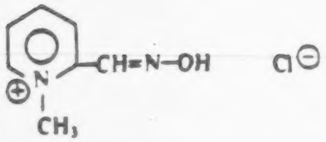
The termination of World War II brought to light the highly potent chemical-warfare agents of the organophosphorus ester class that had been synthesized by both sides during the conflict. The properties, pharmacology, and toxicology of these anticholinesterase agents were discussed in Volume 1 of the National Research Council report.[17] Such agents as diisopropyl fluorophosphate (DFP), sarin (GB), soman (GD), and tabun (GA) were a fascination to pharmacologists who conducted extensive studies in the early postwar years. It soon became evident that no available antidotes could block the pharmacologic activity of these chemicals, alleviate the signs and symptoms of toxicity, or restore normal bodily functions after exposure. Atropine readily antagonized the muscarinic actions, including those in the central nervous system (CNS), but elicited no reversal of the nicotinic effects. Better forms of therapy were sought, particularly to alleviate the nicotinic effects of anticholinesterase agents.

The first suggestion of a practical form of antidotal therapy came in 1949 from Hestrin,[9] who found that acetylcholinesterase (AChE) catalyzed the formation of acetohydroxamic acid when incubated with sodium acetate and hydroxylamine. Critical in vitro studies in the next decade led to the development of a practical approach to therapy. The crucial concept in these studies was the recognition that the compound formed when AChE reacted with a phosphorus ester was a covalent phosphoryl-enzyme intermediate similar to that formed in the hydrolysis of acetylcholine.[23] Wilson and colleagues, beginning in 1951, demonstrated that AChE inhibited by alkyl phosphate esters (tetraethyl pyrophosphate, TEPP) could be reactivated by water, but that free enzyme formed much more rapidly in the presence of hydroxylamine.[20,21] Similar results were obtained by Hobbiger.[10] In 1953, Wilson and Meislich[26] synthesized nicotinehydroxamic acid and the corresponding methiodide; these agents were far more potent reactivators of alkyl phosphate-inhibited AChE than was hydroxylamine. In 1955, Wilson described picolinehydroxamic acid as the best reactivator among a series of quaternary hydroxamic acids, noting that oximes also were reactivators. In in vitro studies, Davies and Green[4] and Childs *et al.*[3] reported that 2-formyl-*N*-methylpyridinium iodide oxime (2-PAM iodide form) was 170 times more active than picolinehydroxamic acid in reactivating TEPP-inhibited AChE in vitro, 30 times more active in reactivating DFP-inhibited AChE, and 400 times more active in reactivating GB-inhibited AChE. Similar findings were reported by Wilson and Ginsburg.[24,25]

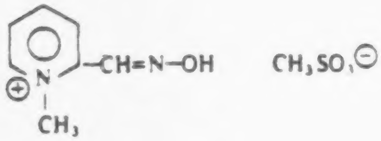
The first investigators to show the marked antidotal properties of pralidoxime compounds (Figure 2-1) were Kewitz and Wilson.[13] Askew[1] noted marked species differences in the rate and effectiveness of oximes and hydroxamic acids in reactivating alkyl phosphate-inhibited AChE. Other in vivo studies demonstrated that some oximes were effective in antagonizing the blockade of neuromuscular transmission after treatment of animals with TEPP, DFP, or GB.[12] The methyl methanesulfonate derivative of 2-PAM, P2S (Figure 2-1), was shown to be very effective by virtue of greater water solubility.[5] Additional studies demonstrated that the most effective treatment of organophosphorus ester-poisoned animals was to use oximes concomitantly with atropine. The general conclusions were that atropine minimizes or abolishes the toxic actions of AChE-inhibiting compounds on muscarinic and central cholinergic sites and the pyridinium and other quaternary hydroxamic acids and oximes reverse the toxic effects of the organophosphorus esters by reactivating the inhibited AChE.[14,19]

Success with the pyridinium oximes led to an intensive search for more effective oximes and the discovery of an especially potent derivative, 1,3-bis(4-formylpyridinium)propane dibromide bisoxime (TMB-4) (Figure 2-1), which reactivated serum-inhibited AChE in vitro more rapidly than 2-PAM or P2S.[11,18] TMB-4 was an effective chemical adjunct to atropine in sarin-poisoned mice, rats, rabbits, cats, and dogs.[2] Further modifications of the bispyridinium structure--insertion of different groups between the pyridinium rings--led to the synthesis of bis(4-formyl-*N*-methylpyridinium oxime) ether bichloride, known originally as LuH6 and later as toxogonin (Figure 2-1).[7] Its advantage was that it was less toxic than TMB-4 and also capable of reactivating AChE inhibited by a variety of organophosphorus esters.[6,15]

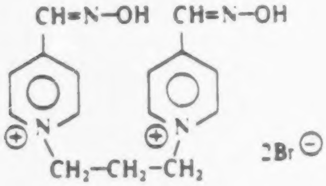
With the demonstration of species variability in response to the various oximes,[1] it was noted that results obtained with a particular organophosphate were not obtained with other agents, even if


CH=N–OH
Cl⊖
N
⊕
CH₃

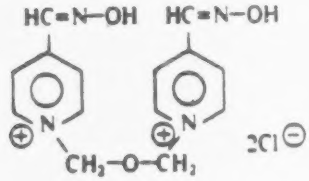
2-PAM. Pyridine-2-aldoxime methiodide methyl chloride


CH=N–OH
CH₃SO₃⊖
N
⊕
CH₃

P2S. Pyridine-2-aldoxime methyl methanesulfonate


CH=N–OH
CH=N–OH
N
N
⊕
⊕
CH₂–CH₂–CH₂
2Br⊖

TMB-4. N,N-trimethylene bis(pyridine-4-aldoxime bromide)


HC=N–OH
HC=N–OH
N
N
⊕
⊕
CH₂–O–CH₂
2Cl⊖

Toxogonin. Bis(4-formyl N-methylpyridinium oxime) ether dichloride

FIGURE 2-1 Structural formulas of cholinesterase reactivators tested at Edgewood

they formed the same phosphorylated enzyme. This meant that screening of the reactivators for suitable antidotal properties necessitated in vivo testing with as many organophosphorus esters as possible. It also meant that data from animal studies could not be extrapolated to humans without considerable caution, necessitating testing in humans. The effectiveness of oximes in treating poisoning by organophosphorus ester chemical-warfare agents stimulated the U.S. Army to initiate studies with four compounds--2-PAM, P2S, TMB-4, and toxogonin--in military volunteers from 1958 to 1975, to assess their effectiveness against low doses of suitable militarily important organosphates. This chapter examines some pertinent features of the pharmacology and toxicology of the pralidoxime reactivators (Table 2-1). It presents conclusions of the Panel on Cholinesterase Reactivators as to the potential of these agents to impair chronically the health and well-being of the volunteers who participated in the studies conducted by the Department of the Army. It focuses on the four agents tested at Edgewood. A detailed report of the pharmacology and toxicology of the four oxime reactivators is in Appendix B.

TABLE 2-1

Subjects Tested with Cholinesterase Reactivators at Edgewood

Tox. No.	EA No.	Compound	CAS NO.	No. Subjects (Approx.)
D-1	2170	2-PAM: pralidoxime chloride (or iodide) protopam chloride (or iodide); 2-formyl-N-methylpyridinium chloride (or iodide) oxime; pyridine-2-aldoxime methochloride (or methiodide)	51-15-0	607
D-2		P2S: methyl methanesulfonate derivative of 2-PAM; pralidoxime methanesulfonate; Protopam methanesulfonate	154-92-2	95
D-3	3475	Toxogonin: LuH6; bis(4-formyl-N-methylpyridinium oxime) ether dichloride	114-90-9	41
D-4	1814	TMB-4; 1,1'-trimethylene-bis(4-formyl-pyridinium) bromide (or chloride) dioxime; trimedoxime; 1,3-bis (4-formylpyridinium)propane dibromide (or dichloride) bisoxime	56-97-3 (bromide) 3613-81-9 (chloride)	32

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REVIEW OF AVAILABLE INFORMATION ON 2-PAM, P2S, TMB-4, AND TOXOGONIN

CHARACTERISTICS

Pralidoxime chloride (EA 2170), $[C_7H_9N_2O]Cl$ (Figure 2-1), is a white, nonhygroscopic, crystalline powder, with a molecular weight of 172.63, a melting point of 226-227°C, and a solubility in water of over 1 g/ml. It is a cholinesterase reactivator and was tested alone and with anticholinesterase chemicals on human volunteers at Edgewood. It is manufactured by Ayerst Laboratories, approved by the FDA, and widely used in the United States as an antidote to alleviate the acute responses to poisoning by organophosphorus insecticides and other anticholinesterase chemicals.[4,11,73]

Pralidoxime methanesulfonate (P2S), $[C_7H_9N_2O]CH_3O_3S$, consists of hygroscopic crystals with a molecular weight of 232.28 and a melting point of 155°C.[11,117] This is reportedly the preferred oxime in the United Kingdom.[73]

Toxogonin (EA 3475), $[C_{14}H_{16}Cl_2N_4O_3]$, is a grayish-white powder that exists as the chloride with a molecular weight of 359.22, a melting point of 229°C, and free solubility in water. A dibromide compound with a melting point of 202°C is also used. This oxime is much used in many European countries.[73]

TMB-4 (EA 1814), $[C_{15}H_{18}N_4O_2]Br_2$, exists as odorless, light tan crystals with a molecular weight of 446.21 and a melting point of 238-241°C and is also available as the chloride.[11, 73] Russian and Yugoslavian investigators have reported the use of TMB-4 as an antidote in anticholinesterase poisoning.[17,73]

All four cholinesterase reactivator compounds are pralidoximes. The first two are monoquaternary oximes; the last two are bisquaternary oximes.

BIOCHEMISTRY

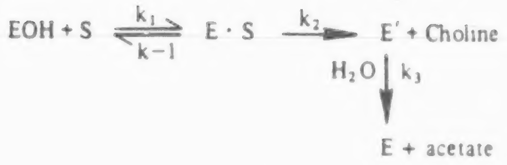
The body contains two main classes of cholinesterase: acetylcholinesterase (EC 3.1.1.7) and butyrylcholinesterase (EC 3.1.1.8).[27] The former, sometimes referred to as true cholinesterase, is mainly a tissue enzyme and is found mainly in such tissues as the synapses of the cholinergic system; it is also found in other tissues, such as erythrocytes, where its function is uncertain. The latter, referred to as pseudocholinesterase, is a soluble enzyme that is synthesized in the liver and circulates in the plasma.

Acetylcholine is the optimal substrate for acetylcholinesterase, whereas butyrylcholine is the optimal substrate for butyrylcholinesterase.[80,104] Acetylcholinesterase does not hydrolyze butyrylcholine very efficiently.

Acetylcholinesterase can be extracted from tissues to various extents by saline buffers, but often a detergent is necessary to solubilize the larger part of the enzyme. Even a single tissue usually yields a number of enzyme forms with different sedimentation coefficients.[72] Some of the forms are highly asymmetric; some tend to aggregate, unless the salt concentration is high. Regardless of the source or the molecular form, the kinetic properties of acetylcholinesterase are similar. Although it contains a number of catalytic subunits, there are no homotropic allosteric effects, nor do there appear to be any physiologic regulators of its activity.

Acetylcholinesterase is subject to substrate inhibition at high concentrations, but Michaelis kinetics are observed at lower concentrations, because the substrate constant and the Michaelis constant differ by a factor of 100. Turnover numbers run about 2-9 x 10^5 min^{-1}, and K_m (Michaelis constant) values are about 0.2 mM.[76,116] Whatever the source, the enzyme is subject to inhibition by the same reversible and irreversible inhibitors. Most of the kinetic work has been done with the saline-extracted 11S enzyme from electric eel and the detergent-extracted 6S enzyme from erythrocytes. The former is a tetramer derived from the native enzyme by the action of proteases; the latter is a dimer.[89]

The following diagram illustrates the reaction of acetylcholinesterase (E) with acetylcholine (S) to produce an acetylenzyme intermediate (E') with later hydrolysis of the intermediate and regeneration of the enzyme.[113, 114] K_M is calculated as shown in the diagram. The catalytic constant, k_{cat}, refers to the overall decomposition of the enzyme intermediate (E.S, E').



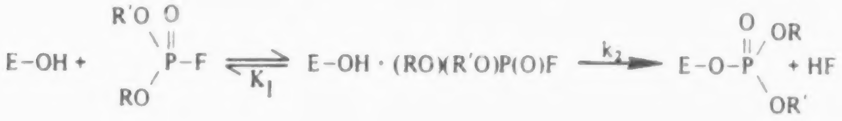
$$EOH + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E\cdot S \xrightarrow{k_2} E' + \text{Choline}; \quad E' \xrightarrow[H_2O]{k_3} E + \text{acetate}$$

$$k_M = \frac{k_{-1} + k_2}{k_1} \bigg/ \left(1 + \frac{k_2}{k_3}\right)$$

$$k_{cat} = k_2 \bigg/ \left(1 + \frac{k_2}{k_3}\right)$$

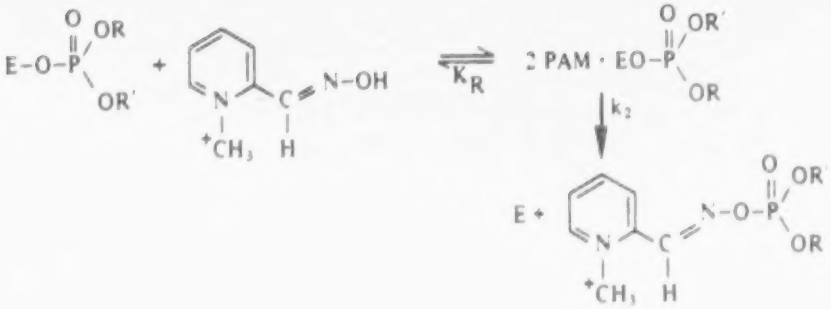
In acetylation, the enzyme acts as a nucleophile, and choline as a leaving group; in deacetylation, water is the nucleophile, and the enzyme is the leaving group. The enzymic nucleophile is the hydroxyl group of a specific serine residue. The mechanisms of both acetylation and deacetylation probably involve the formation of tetrahedral intermediates. The active site contains an imidazole group that functions first as a general base and than as a general acid in each step and whose ionization determines in part the pH variation of enzyme activity.

Details of enzyme-substrate and enzyme-inhibitor reactions were described in Volume 1 (p. 8). In summary, organophosphate esters with good leaving groups phosphorylate the enzyme by a mechanism similar to acetylation of the enzyme.



$$E{-}OH + (RO)(R'O)P(O){-}F \underset{K_I}{\rightleftharpoons} E{-}OH\cdot(RO)(R'O)P(O)F \xrightarrow{k_2} E{-}O{-}P(O)(OR)(OR') + HF$$

These substances are sometimes called irreversible inhibitors, because the hydrolysis of the phosphorylated enzyme by water is slow. Various nucleophiles containing a substituted ammonium group will dephosphorylate the phosphorylated enzyme much more rapidly than water. This was recognized early in the quest for a practical reactivating agent when choline was found to be a reactivator. Because a hydroxyl group in an alcohol is a weak nucleophile at neutral pH, the capacity of choline to function as a reactivator must be a consequence of its molecular complementarity with the enzyme and the increased acidity of the alcohol. The idea arose of finding a rigid structure that would include a quaternary ammonium group and an acidic nucleophile that would be complementary with the phosphorylated enzyme in such a way that the nucleophilic oxygen would be positioned close to the electrophilic phosphorus atom. This led to the pralidoxime compounds (PAMs). The syn isomer of 2-PAM (2-PAM-syn) turned out to be especially active. The reaction of 2-PAM with the phosphorylated enzyme is shown in the following diagram.



The pseudo-first-order rate constant for reactivation of 11S enzyme is:

$$k\,(\text{pseudo-first-order}) = \frac{k_2}{1 + \frac{K_R}{2\text{-PAM}}}$$

When the oxime concentration is smaller than K_R ($[2\text{-PAM}] \ll K_R$), the reactivation follows second order kinetics, and the rate constant can be described as shown below.

$$k\,(\text{second-order}) = \frac{k_2}{K_R}\,(2\text{-PAM})$$

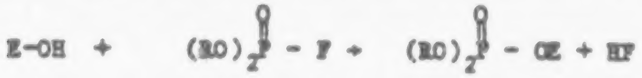
The value for K_R as determined for eel cholinesterase is about 10^{-4} M, and that for k_2, about 4 min^{-1}, so the second-order rate constant, k_2/K_R, is 4×10^4 M^{-1} min^{-1}.

Thus, the biochemical characteristics affecting the reactivation of cholinesterase are complex and only partially understood. Knowledge of the kinetics of the various rate-determining processes is essential to the understanding of the inhibitor-reactivation process.

MECHANISMS OF ACTION

The therapeutic action of oximes resides largely in their capacity to reactivate cholinesterases without contributing markedly toxic actions of their own at recommended or usual doses. Other actions may contribute to their effectiveness: they may react directly with the anticholinesterase agent,[41,45] block its reaction with cholinesterase,[90] modulate the release of acetylcholine,[30,74] block acetylcholine's agonistic activity (cholinolytic effect),[15,35,51] or increase excretion of the anticholinesterase agent.[48,86] Some of the other possible mechanisms are discussed later, but the interaction between oxime and free or unbound organophosphorus ester is considered below.

The basic reaction believed to occur in the inhibition of acetylcholinesterase by some alkylphosphates is shown in Reaction 1.

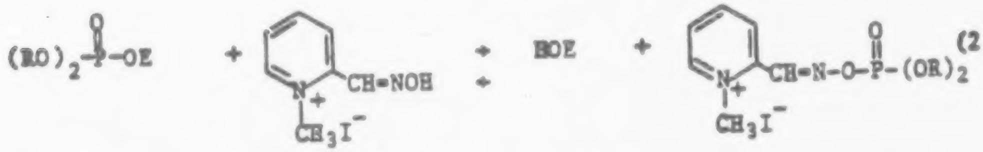


$$E\text{-}OH + (RO)_2\overset{O}{\overset{\|}{P}} - F \rightarrow (RO)_2\overset{O}{\overset{\|}{P}} - OE + HF \qquad (1)$$

active enzyme inhibitor inhibited enzyme

The inhibited enzyme is stable in aqueous environments, and lethality results from a blocking of transmission at cortical cholinergic synapses, such as those involved in breathing.

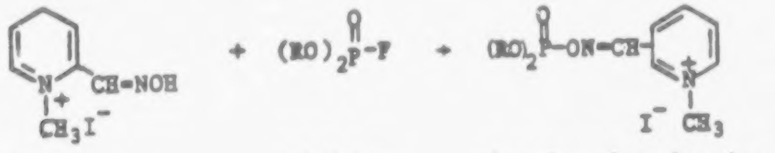
The reactivation of the inhibited enzyme can be achieved with selected oximes (such as 2-PAM), as shown in Reaction 2.



(2)

inhibited enzyme reactivator active enzyme phosphorylated oxime

The reactivation is an equilibrium reaction. Under some conditions--e.g., when the oxime reacts directly with the organophosphate ester (Reaction 3) or when the equilibrium constant is less than 1 (Reaction 2)--the phosphorylated oxime can inhibit the enzyme by driving the reaction to the left.



(3)

reactivator inhibitor phosphorylated oxime

Thus, the phosphorylated oxime can itself be a potent cholinesterase inhibitor. Wilson and Ginsburg advanced this concept when they noticed incomplete reactivation of acetylcholinesterase by 2-PAM and suggested that the phosphorylated oxime reinhibited the enzyme (according to Reaction 2).

Studies of the in vitro reaction between the alkaline oximes and sarin (isopropyl methylphosphonofluoridate, GB) revealed that 2-PAM reacted rapidly with the organophosphorus ester in solution at physiologic pH and temperature (Figure 2-2).[43]

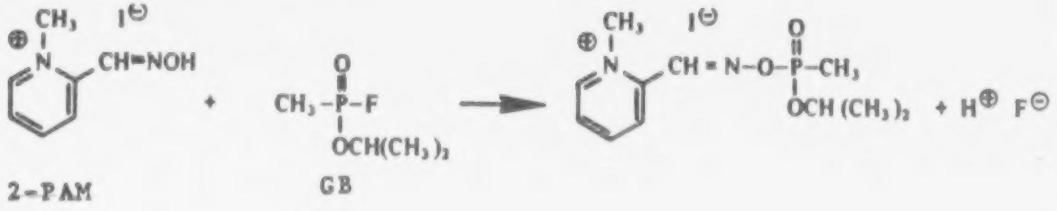


FIGURE 2-2 Suggested interaction between 2-PAM and GB (isopropyl methylphosphonofluoridate). Reprinted with permission from Hackley et al.[45]

Many of the oximes reacted in two distinct acid-producing steps: an initial phosphonylation of the oxime and then a breakdown into secondary products.[44] Although the phosphonylated intermediate of 2-PAM could not be prepared, the phosphonylated derivative of 4-PAM was synthesized and found to be a potent inhibitor of eel acetylcholinesterase and to be toxic to mice, having an intravenous LD_{50} of 0.2 mg/kg of body weight.[44] Green and Saville found that MINA (monoisonitrosoacetone or 1,2-propanedione 1-oxime) underwent a stoichiometric reaction with sarin.[41] Additional studies confirmed that 2-PAM, 4-PAM, and probably TMB-4 were converted to potent inhibitors of AChE when they reacted with sarin in vitro.[45] That TMB-4 was converted to a potent anticholinesterase agent when it reacted with sarin in vitro was confirmed in studies that suggested that this bis-quaternary oxime could exist either as a monophosphonylated or as a diphosphonylated compound (Figure 2-3).[64] Zech et al. demonstrated that 2-PAM, 4-PAM, and TMB-4 were more potent inhibitors of cholinesterases after incubation with dimethoate (O,O-dimethyl S-methylcarbamoylmethyl phosphorodithioate) or derivatives.[119] Later studies, in which sarin was incubated with P2S and toxogonin, demonstrated that unstable phosphonylated oximes were formed, but that these intermediates were potent inhibitors of cholinesterase.[26]

Although it appears that these phosphonylated oximes rapidly degrade in aqueous solutions, it has been suggested that they can have a harmful effect on the oxime therapy of poisoning by some organophosphates if they are formed in vivo, react with excess

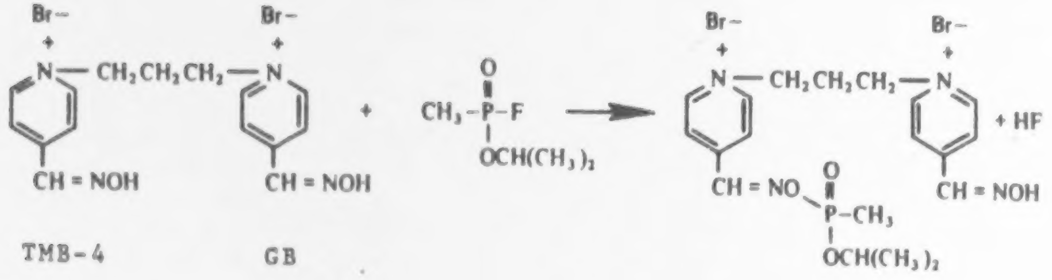


FIGURE 2-3 Interaction between TMB-4 and GB to form the monophosphonylated oxime. Reaction with a further molecule of GB would form the diphosphonylated compound.

("free") circulating ester in cases of severe poisoning, and create a new inhibitor as potent as or more potent than the original anticholinesterase. The inhibitory potency of phosphonylated oximes has been estimated from their effects on the course of the reactivation of phosphonylated AChE when they were formed during the reaction.[2,91] Thus, it appears that the potential for the phosphorylated oximes to inhibit cholinesterases in vivo is substantial, but depends entirely on the stability of these intermediates in the body.

A practical limitation on the usefulness of reactivators comes about because cholinesterase inhibited by organophosphates undergoes a secondary reaction termed "aging." This phenomenon is discussed in Volume 1 of this report series. In brief an alkyl group is cleaved from the phosphorylated enzyme, leaving an "aged" enzyme that is resistant to reactivation by the oxime. The rate at which a phosphorylated enzyme "ages" is determined by the alkyl groups on the organophosphate, as described in Volume 1.

PHARMACOKINETICS

The pharmacokinetic features of these drugs, based on the evidence produced and the conclusions drawn by various investigators, are described in Appendix B. This section touches on the highlights and restates the material from another viewpoint.

Two features of the cholinesterase reactivating agents are critical for their pharmacokinetics:

Oxime reactivators (R-CH=NOH) are weak acids that partly ionize at biologic pH. This property allows them to function as nucleophiles and displace organophosphate moieties from inhibited acetylcholinesterase. It also makes them vulnerable to decomposition by other mechanisms in the body.

The oximes contain a quaternary ammonium group that contributes to their acidity and their strong binding to the inhibited enzyme. This appears to be a key structural element in known reactivators, but it tends to make them poorly soluble in lipids. Practically, this means that the drugs are slowly absorbed from the gastrointestinal tract, have difficulty entering the brain, do not easily enter hepatic cells to be biotransformed, and are not reabsorbed from the renal tubular urine.

2-PAM has been studied extensively by pharmacokinetic means, and data are available on its absorption, tissue distribution, blood concentration, and elimination by humans and animals. Although it has been given as various salts, the particular form administered is of no consequence once the material is absorbed and dissociated into the cation by body fluids.[61,100,106]

2-PAM is poorly and variably absorbed from the human gastrointestinal tract; only 10-50% of an oral dose is recovered in urine unchanged or as metabolites.[58,61,99,100,106] The absorption occurs, at least in dogs, in a segment of the ileal-jejunal area[68] either by passage through the small membrane pores in this section or by some more active absorption process that carries a variety of quaternary ammonium compounds into the blood. The overall absorption rate is lower than the elimination rate; that is, absorption is the rate-limiting step. Hence, blood concentrations are always low after oral administration, and substantial blood concentrations are difficult to produce or maintain when the drug is given by this route.[28,99] Marked gastrointestinal distress is produced by orally given oximes, particularly if therapy is continued.[99] 2-PAM may effectively be given either intravenously or intramuscularly, although the latter route is more erratic and slower in producing high blood concentrations and may involve some local pain.[96]

Like most quaternary ammonium compounds, 2-PAM is distributed mainly to extracellular water, and some tissue sequestration occurs.[100] 2-PAM is only weakly bound to plasma proteins.[34,100]

2-PAM crosses the blood-brain barrier with difficulty. 2-PAM in rat brain, 10 min after injection, is only about 5-12% of that in plasma; higher percentages are in the more heavily vascularized areas, such as cerebral and cerebellar cortex and inferior colliculi.[34] This low brain-to-blood ratio persists, but over the next 6 h the brain and blood come closer to equilibrium as the blood concentration decreases sharply.[34] The low brain concentration is puzzling, inasmuch as the oximes depress anticholinesterase-induced seizures and void the part of the weakness of respiratory muscle contractions that is thought to be due in part to the action of organophosphate in the brain by suppressing anticholinesterase inactivation of central respiratory drive.[34] Hypoxia might cause disruption of the blood-brain barrier, thereby allowing the oxime to enter the brain. Some anticholinesterases may also facilitate the entry of oximes into the central nervous system.[34] Regardless of mechanism involved, oximes can cause remarkable improvement in CNS signs and symptoms in some patients. There is some evidence that peripheral actions, such as might occur at neuromuscular junctions, could contribute to muscle weakness.[60,70,88]

2-PAM is eliminated rapidly by man and animals. In humans, biologic half-life is about 1-2 h.[100] This short half-life is due in part to metabolism, but more to the fact that renal clearance approaches 700 ml/min,[100] i.e., almost that of p-aminohippuric acid (PAH). Investigators have therefore suggested that 2-PAM might be secreted into the urine.[14,50,100,108]

Interpretation of data on renal elimination is complicated by the presence of a quaternary group on one end of the oxime molecule and a weak acid at the other (Figure 2-1). They imply different mechanisms of renal handling. Swartz and Sidell [108] showed that in human beings both chemical groups are important. The more important of the two actions is the active secretion of the compound by the organic-base-secreting system of the kidney. This process can be slowed by one-third by coadministering high doses of thiamine.[57] Das Gupta et al.[25] claimed that the inhibiting effect of thiamine occurs in female, but not in male, rats. The net clearance of 2-PAM approaches that of PAH,[100] but is not as great; hence there may be some simultaneous active reabsorption of the material.[50] Like most cations, 2-PAM in the urine does not readily diffuse back from renal tubule to blood. The process here is complicated, in that the oxime has a pKa of 7.8-8.[108] It is more ionized at lower pH, and the renal loss is inversely proportional to pH.[14] Although the pH/pKa relation and active absorption contribute to the resulting or biologic half-life, active secretion into the urine is the predominant feature, and the total half-life is correspondingly short. Because of this, it is difficult to accumulate and maintain a substantial concentration of material.

The rapid renal elimination does not leave much time for metabolism of 2-PAM; about 90% of injected material is recovered unchanged in urine.[100] Nonetheless, 2-PAM is metabolized by liver, primarily by removal of the oxime moiety, and a number of metabolites have been identified,[32,78,111,112] some of them in rat brain, plasma, and urine.[34]

The blood concentrations, time-courses, and half-lives (1.2-1.4 h) of 2-PAM and toxogonin are similar in humans,[100] but toxogonin has a smaller volume of distribution than 2-PAM (174 vs. 795 ml/kg) and a smaller renal elimination (clearance, 133 vs. 717 ml/min). The smaller volume of distribution and slower clearance imply different distribution and handling of the two oximes. They also imply that, if toxogonin and 2-PAM were given in equimolar concentrations, the toxogonin plasma concentration would be greater than that of 2-PAM.

TMB-4 is unstable in fecal matter in vitro; only about 3% of an oral dose is found in urine and 2% in feces.[61]

ADVERSE EFFECTS

The adverse effects of various oximes (including diacetylmonoxime, which was not used at Edgewood) in humans are listed in Tables 2-2 through 2-7. These tables list only significant findings.

Although Hopff and Waser[54] have listed mechanisms of action whereby reactivators of inhibited cholinesterase could be harmful, the real cause of most of the observed adverse effects is obscure. One exception is the slight inhibition of cholinesterase caused by a single dose of TMB-4 (dibromide form).[61]

Regardless of the underlying mechanism, the observed adverse effects are usually mild and brief. Many last only a few minutes; almost all disappear within a few hours after a single dose. A number of investigations, especially those concerning metabolism or pharmacokinetics, revealed no adverse effects, even though they were looked for.[31,101,107] Gastrointestinal disturbance after oral administration of oximes, especially P2S and TMB-4 chloride, may be severe enough to require discontinuation.[20] But doses of P2S as high as 8 g caused no symptoms (including gastritis) when given as tablets coated with dimethylaminoethyl methacrylate, each containing 400 mg of the drug and 71 mg of excipient.[100]

Local pain and increased creatine phosphokinase in the blood follow intramuscular injection. Using 2-PAM chloride and saline, Sidell et al.[95] showed that the degree of tissue injury was directly related to the osmolarity of the injected solutions when the volume was kept constant and directly related to the volume when the osmolarity was constant. However, under identical conditions, 2-PAM chloride was somewhat more injurious than saline.

Hepatic injury has been recorded in connection with 2-PAM (chloride), TMB-4 (chloride) and toxogonin. In the case of 2-PAM chloride, hepatic injury was manifest by transient increases in SGPT

TABLE 2-2

Adverse Effects Observed in Humans After Administration of 2-PAM Iodide

Effects	Dose and Route	References
Taste (bitter, metallic, salty, or mushy)	b	
Iodism	≥5 g, po	61
Local irritation	≥1 g, iv, NEG	56
Headache	≥1 g, iv	56
Dizziness and nausea, possibly progressing to vomiting	≥1 g, iv	56
Blurred vision, impaired accommodation	≥1 g, iv	56
Muscular weakness and/or malaise, fatigue, etc.	b	
Gastrointestinal distress	b	
Paresthesia and/or anesthesia	b	
Rash	b	
Bleeding	b	
Hypertension (moderate systolic and diastolic with increased pulse pressure and tachycardia)	≤10 g, po, NEG ≤1 g, iv, NEG	61 56
Initial hypertension followed by hypotension	b	
ECG changes	b	
Icterus and/or clinical test implicating liver	b	
Other biochemical changes	≥1 g, iv, NEG	56
Loss of consciousness	b	
Convulsions	b	

---

[a] po = oral; iv = intravenous; NEG = administration failed to produce effect indicated.

[b] Effect looked for, but not observed at any dose or by any route.

TABLE 2-3

Adverse Effects Observed in Humans After Administration of 2-PAM Chloride

Effects	Dose and Route[a]	References
Taste (bitter, metallic, salty, or mushy)	b	
Iodism	b	
Local irritation	0.6 g, im	101
	≥0.175 g, im	101
Headache	≥2 g, iv	20
Dizziness and nausea, possibly progressing to vomiting	b	
Blurred vision,	≥2 g, iv	20
impaired accommodation	0.25 g, iv	108
Muscular weakness and/or malaise, fatigue, etc.	b	
Gastrointestinal	≥8 g, po	99
distress	≥2 g, po qid, 2 d	99
	2.1 g, iv	20
	2 g, po, bid, 180 d NEG	20
Paresthesia and/or anesthesia	b	
Rash	b	
Bleeding	b	
Hypertension (moderate	≤3.7 g, po, NEG	61
systolic and diastolic	≤4 g, po, NEG	20
with increased pulse	2 g, iv	20
pressure and tachycardia)	2 g, iv	20
	1 g, iv, NEG	20
	≤2 g, po, qid, 3 d, NEG	20
	2 g, po, bid, 180 d, NEG	20
	0.35 g, iv, NEG	108
Initial hypertension followed by hypotension	b	
ECG changes	3 g, iv	20
	2 g, po, bid, 180 d	
Icterus and/or clinical test implicating liver	2 g, po, bid, 180 d	20
Other biochemical	≤3 g, iv, NEG	20
changes	≤2 g, po, qid, 3 d, NEG	20
Loss of consciousness	b	
Convulsions	b	

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[a] in = intramuscular; iv = intravenous; po = oral; qid = 4 times a day; bid = 2 times a day; NEG = administration failed to produce effect indicated.

[b] Effect looked for, but not observed at any dose or by any route.

TABLE 2-4

Adverse Effects Observed in Humans After Administration of P2S

Effects	Dose and Route[a]	References
Taste (bitter, metallic, salty, or mushy)	b	
Iodism	b	
Local irritation	≥0.7 g, im 0.5 g, im	6 52
Headache	b	
Dizziness and nausea, possibly progressing to vomiting	b	
Blurred vision, impaired accommodation	0.5 g, im, NEG 1 g, po, qid + 0.5 g, im	52 52
Muscular weakness and/or malaise, fatigue, etc.	b	
Gastrointestinal distress	≥2.5 g, po, bid, 180 d	20
Paresthesia and/or anesthesia	b	
Rash	b	
Bleeding	b	
Hypertension (moderate systolic and diastolic with increased pulse pressure and tachycardia)	≤5 g, po, NEG 3 g, po, NEG 3g, iv	61 20 20
Initial hypertension followed by hypotension	b	
ECG changes	3 g, po, NEG 3 g, iv 2.5 g, po, bid, 77 d	20 20 20
Icterus and/or clinical test implicating liver	b	
Other biochemical changes	b	
Loss of consciousness	b	
Convulsions	b	

---

[a] im = intramuscular; po = oral; iv = intravenous; qid = 4 times a day; bid = 2 times a day; NEG = administration failed to produce effect indicated.

[b] Effect looked for, but not observed at any dose or by any route.

TABLE 2-5

Adverse Effects Observed in Humans After Administration of TMB-4 Dichloride

Effects	Dose and Route[a]	References
Taste (bitter, metallic, salty, or mushy)	b	
Iodism	b	
Local irritation	b	
Headache	b	
Dizziness and nausea, possibly progressing to vomiting	≥1 g, po, 70 d	20
Blurred vision, impaired accommodation	b	
Muscular weakness and/or malaise, fatigue, etc.	≥1 g, po, 70 d	20
Gastrointestinal distress	≥1 g, po, 70 d	20
Paresthesia and/or anesthesia	≥1 g, po, 70 d	20
Rash	≥1 g, po, 70 d	20
Bleeding	≥1 g, po, 70 d	20
Hypertension (moderate systolic and diastolic with increased pulse pressure and tachycardia)	b	
Initial hypertension followed by hypotension	4 g, po 2 g, iv	20
ECG changes	4 g, po, NEG 2 g, iv	20
Icterus and/or clinical tes implicating liver	≥1 g, po, 70 d	20
Other biochemical changes	b	
Loss of consciousness	b	
Convulsions	b	

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[a] po = oral; iv = intravenous; NEG = administration failed to produce effect indicated.

[b] Effect looked for, but not observed at any dose or by any route.

TABLE 2-6

Adverse Effects Observed in Humans After Administration of Toxogonin Chloride

Effects	Dose and Route[a]	References
Taste (bitter, metallic salty, or mushy)	0.175 g, im	97
	≥3 g, po	98
	≥1.84 g, po	102
Iodism	b	
Local irritation	≥0.175 g, im	97
	0.25 g, im	33
Headache	≥2.76 g, po	102
Dizziness and nausea, possibly progressing to vomiting	7 g, po	98
Blurred vision, impaired accommodation	b	
Muscular weakness and/or malaise, fatigue, etc.	≥2.76 g, po	102
Gastrointestinal distress	b	
Paresthesia and/or anesthesia	0.175 g, im	97
	≥3 g, po	98
	0.25 g, im	33
	≥2.76 g, po	102
Rash	b	
Bleeding	b	
Hypertension (moderate systolic and diastolic with increased pulse pressure and tachycardia)	0.175 g, im	97
	≤4.6 g, po, NEG	102
Initial hypertension followed by hypotension	b	
ECG changes	b	
Icterus and/or clinical test implicating liver	0.25 g, im, 2 d, NEG	16
	≤4.6 g, po, NEG	102
Other biochemical changes	b	
Loss of consciousness	b	
Convulsions	b	

---

[a] im = intramuscular; po = oral; NEG = administration failed to produce effect indicated.

[b] Effect looked for, but not observed at any dose or by any route.

TABLE 2-7

Adverse Effects Observed in Humans After Administration of Diacetylmonoxime

Effects	Dose and Route[a]	References
Taste (bitter, metallic, salty, or mushy)	≥1 g, iv	56
Iodism	b	
Local irritation	≥1 g, iv ≥0.5 g, iv	56 42
Headache	b	
Dizziness and nausea, possibly progressing to vomiting	≥1 g, iv ≥0.5 g, iv	56 42
Blurred vision, impaired accommodation	≥1 g, iv	56
Muscular weakness and/or malaise, fatigue, etc.	b	
Gastrointestinal distress	b	
Paresthesia and/or anesthesia	≥1 g, iv ≥0.5 g, iv	56 42
Rash	b	
Bleeding	b	
Hypertension (moderate systolic and diastolic with increased pulse pressure and tachycardia)	b	
Initial hypertension followed by hypotension	b	
ECG changes	b	
Icterus and/or clinical test implicating liver	b	
Other biochemical changes	b	
Loss of consciousness	≥1 g, iv	56
Convulsions	≥1 g, iv	56

---

[a] iv = intravenous.

[b] Effect looked for, but not observed at any dose or by any route.

and SGOT in 4 of 29 subjects, positive thymol flocculation test in 4 subjects, and positive urobilinogen test in 11 subjects. This occurred when substantial oral doses (2 g twice a day) were administered for 6 mo. In the case of TMB-4 chloride, hepatic enzyme changes were accompanied by icterus, petechial bleeding, and prothrombin times of 40% and 50% of normal, respectively, in two of 11 subjects. All adverse effects were marked by the time TMB-4 chloride had been administered for 6 wk orally at increasing rates of 0.8-2.3 g. All symptoms except icterus subsided completely in 1 or 2 wk after administration was stopped; clearing of icterus required 3-6 wk. The investigators considered the icterus similar to that produced by chlorpromazine.[20]

GASTROINTESTINAL EFFECTS

Direct effects of oximes on the gastrointestinal tract are related entirely to the route of administration--they are seen only after oral administration. Ingestion of tablets of 2-PAM or related salts is often accompanied by transient diarrhea and cramps. The full explanation is not known. In the case of P2S, it has been suggested that a dose of 20 mg/kg inhibits ATPase and therefore produces excess intestinal fluid and diarrhea. The effect depends on concentration and is reversible. 2-PAM has no effect on cyclic AMP (cAMP), adenylate cyclase, or phosphodiesterase.[66]

Repeated oral administration of 2-PAM or TMB-4 in dogs may lead to areas of acute necrosis and to deposition of fibrous tissue in the stomach.[1] Whether those effects are due to a specific action of the oxime or to chronic irritation related to repeated administration is not known. Nor are the long-term effects of such chronic administration and consequent fibrosis known. However, single doses, even orally, have not been reported to produce tissue changes. No change was found in the gastrointestinal tracts of rats given P2S (200 mg/kg) orally each day for 50 d.[23]

Large doses of 2-PAM or toxogonin (at 7100 mg/kg) cause death that may be associated with acute enteritis,[37] but small doses do not seem to produce tissue changes.[23] With small doses, enteritis is not sufficiently severe to cause death.

CARDIOVASCULAR EFFECTS

The adverse effects of cholinesterase reactivating chemicals on the human cardiovascular system are shown in Tables 2-2 through 2-7. These effects may be classified as hypertension characterized by moderate increased systolic and diastolic pressure with hypertension followed by hypotension, and ECG changes.[20,61,97,102,107] The occurrence of adverse cardiovascular effects depends on dosage and route of administration. In general, these effects were seen when large doses of the cholinesterase reactivators were given intravenously or intramuscularly or after repetitive oral doses. Although therapeutic doses of toxogonin produced no effects on heart rate or blood pressure in humans, 2-PAM caused a significant increase in cardiac output in dogs.[21] 2-PAM increases blood pressure in experimental animals and humans.[18,20,38,60,67,103,118] It causes an increase in mycocardial contractility in experimental animals[85] and cardiac arrythmias when used in humans to treat organophosphorus insecticide poisoning.[18,24]

The mechanisms by which 2-PAM exerts its cardiac effects have been studied in experimental animals. At least three classes of action have been attributed to the effects of altered calcium metabolism on autonomic ganglia. A sympathomimetic action of 2-PAM was postulated to explain the increase in blood pressure and the augmented myocardial contractility by one or more of the following mechanisms: 2-PAM may not block the release of the endogenous compounds, but may prevent the uptake of catecholamine;[10] it may stimulate the release of norepinephrine;[18] it increases myocardial contractility by directly stimulating beta receptors;[38] and it increases blood pressure by directly stimulating alpha receptors.[85]

2-PAM increased the contractile force of stimulated rabbit atria that did not result from an increase in the frequency response.[21] Similar results were obtained in rabbits pretreated with reserpine, a catecholamine-depleting agent. These findings are in agreement with previous reports that the cardiovascular effects of 2-PAM on anesthetized dogs were not due to a release of tissue catecholamine.[38,103] Other studies, however, have shown that 2-PAM affects blood pressure in vivo by changing the release or metabolism of endogenous norepinephrine.[10,118]

Most attention has been focused on adrenergic receptors. Recent study of the mechanism of the effect of 2-PAM on cardiac action in rabbit atria indicated that 2-PAM exerts a direct action on vascular smooth muscle that is not mediated through stimulation of the alpha receptors.[21] These results have led to the conclusion that the effect of 2-PAM on the cardiovascular system cannot be explained entirely by its sympathomimetic effect. This conclusion was supported by the finding that 2-PAM caused an increase in the contractile force of isolated aortic strips from rabbits and the finding that phentolamine, an alpha-adrenergic blocking drug, did not affect this response of the aorta. These results contradict the previous finding that phenoxybenzamine, an adrenergic blocking drug, decreased the pressor effect of 2-PAM.[118] The latter finding may be explained by the inhibiting effect of phenoxybenzamine on the drug-induced calcium movement in vascular smooth muscle.[94]

Conflicting results have been reported in regard to the involvement of beta receptors in the mechanism of the inotropic effect of 2-PAM. It was concluded that the direct stimulation of the myocardium by 2-PAM that could be blocked by dichloroisoproterenol (DCI) was responsible for the increase in blood pressure.[38] A recent report, however, has indicated that the increase in blood pressure caused in the dog by 2-PAM was not blocked by propranolol.[103] Similar results were obtained with rabbit atria in vitro[21] in a study that also showed that 2-PAM augments muscle contractility and alters calcium movement.

The studies reported above implicated calcium movement in the mechanism.[21] It was postulated that 2-PAM may increase the rate of calcium movement either by interfering with calcium binding to its binding site or by directly stimulating the release of bound calcium.[21] It was suggested that 2-PAM may not have increased cell permeability or affected the rate of re-entry of calcium into the cell, or 2-PAM may have increased calcium distribution in the vicinity of myofilaments.

Studies of the efflux of ^{45}Ca by stimulated rabbit atria have characterized three calcium pools. Phase I may represent extracellular washout of the ^{45}Ca that binds to the surface of muscle membrane and is characterized by a high rate constant. Phase II may represent loosely bound calcium present in cell membrane and calcium released at the sarcoplasmic reticulum. Calcium in this pool is directly related to contractility.[65,84,93] Phase III may represent the tightly bound calcium that exchanges very slowly and does not play a role in maintaining calcium concentrations. Recent study has shown that the storage or release of calcium at the sarcoplasmic reticulum and other loosely bound calcium sites (cell membrane) that are involved in muscle contractility can be directly affected by 2-PAM.[21] These results indicate that 2-PAM increases the rate of release of Phase II calcium.

It has been postulated that 2-PAM exerts its cardiac action in rabbit atria through its alteration of calcium metabolism. The relaxation phase of skeletal muscle contraction seems to be directly affected by the sarcoplasmic reticulum because of its ability to sequester calcium actively.[29,46] A similar role has been suggested for the sarcoplasmic reticulum in cardiac muscle.[46,83] The onset of muscle contraction takes place when calcium reaches a critcal concentration. This contraction is later reduced by the increased calcium-sequestering activity of the sarcoplasmic reticulum. Thus, 2-PAM can affect this process by decreasing the rate of calcium uptake by the sarcoplasmic reticulum, which results in increasing the time required to reduce the calcium concentration enough to allow relaxation to take place. This was demonstrated by the increase in the relaxation phase. It was suggested that this delay in binding of calcium to the sarcoplasmic reticulum may increase the active state that increased the contractile force.

2-PAM either activates or blocks autonomic ganglia, depending on dose and route and speed of administration. The changes in ganglionic function induced by 2-PAM will be reflected in sympathetic and parasympathetic activity, which may cause changes in cardiovascular functions. These are discussed further in the next section.

NEUROPHARMACOLOGY

The four oximes have, in addition to reactivation of phosphorylated AChE, a variety of actions that can be demonstrated in the absence of previous exposure to organosphosphorus compounds. Experiments that involved only short-term exposure to high concentrations of 2-PAM showed the following short-lived and reversible actions of oximes in nervous tissues: inhibition of AChE, agonist-antagonist effect on cholinergic receptors, ganglionic blockade, and presynaptic actions, such as modification of the release of acetylcholine (ACh) from the nerve terminal.

Effects of Oximes on AChE Activity

ACh contraction of frog rectus abdominis muscle was potentiated by 2-PAM, and progressive AChE inhibition was seen with increasing concentrations of 2-PAM. 2-PAM (at 1.5×10^{-3} M) produced a 66% inhibition.[36] This is far higher than the concentration that would be produced in vivo by administration of ordinary doses. Similar effects were seen on isolated rabbit intestine and atrium[15] and on chicken muscle.[22] In phrenic nerve diaphragm preparations of rat, 2-PAM increased the neuromuscular block induced by depolarizing blocking agents and antagonized the nondepolarizing block induced by curare.[36]

Receptor Interactions

Receptor interactions were seen at nicotinic and muscarinic receptors. No correlations were found, however, between atropine-like or curare-like actions of these compounds and their protective effects against organophosphate poisoning.

Nicotinic Receptor Interactions. After producing an initial brief augmentation of the twitch response and fasciculations,[63] 2-PAM and TMB-4 at 1-20 mg/kg abolished the indirectly stimulated twitch response of skeletal muscle, but did not alter the response to direct stimulation. Neostigmine and edrophonium antagonized, whereas d-tubocurarine increased these effects, which are due to competition for nicotinic acetylcholine receptor sites.[9] Both agents antagonized the response to decamethonium and reduced the effects of carbamoylcholine.[36]

Muscarinic Receptor Interactions. Excitatory muscarinic effects, such as temporary stimulation of salivation and stimulation of intestinal peristalsis, were seen with 2-PAM. Atropine-like actions were seen at high concentrations (15-20 mg/kg or more), and, when injected rapidly, 2-PAM caused temporary diplopia (nicotinic block) and loss of accommodation in the eye.[56] Both TMB-4 and 2-PAM blocked bradycardia induced by vagal stimulation. At low concentrations, neither compound affected normal intestinal peristalsis, but they did block peristalsis caused by increased vagal stimulation. TMB-4, 2-PAM, and toxogonin antagonized the effect of acetylcholine, acetyl- -methylcholine, and other agonists on isolated guinea pig ileum.[62]

Ganglionic Blocking Action

Doses of 2-PAM larger than 40 mg/kg, as well as TMB-4 and toxogonin, produced a temporary block of the cardiac response to vagal stimulation and of the nictitating membrane response to preganglionic, but not postganglionic, stimulation. There was transient hypotension due to block of ganglionic transmission.[9,63,71]

Presynaptic Effects of Oximes

Concentration-dependent presynaptic effects of 2-PAM on the release of acetylcholine from terminals of nerves innervating the rat diaphragm muscle were seen; at concentrations of 10^{-4}-10^{-3} M 2-PAM stimulated the release of acetylcholine; higher concentrations led to a total block of the evoked release of acetylcholine.[30,40]

MUTAGENIC, REPRODUCTIVE, AND CARCINOGENIC EFFECTS

No information on mutagenic, carcinogenic, reproductive, or teratogenic effects of any of the compounds in question is available.

None of the compounds or their in vivo intermediates is likely to bind covalently with DNA and other macromolecules. Other ways of binding with DNA, such as intercalation, cannot be ruled out. There is some membrane transport, albeit limited. It is not possible, therefore, to conclude that they are not mutagens, teratogens, or carcinogens.

THERAPEUTIC USE

Little information is available on the treatment of humans exposed to military agents. However, pralidoxime is an FDA-approved marketed drug in the U.S. and there is substantial experience with therrapeutic use on civilians exposed to agricultural organophosphorus products.

That poisoning by diethoxy compounds can be treated effectively by oximes is nearly universally agreed.[55,59,82,87,92,109] There is general agreement that the therapeutic effects of oximes are less spectacular in poisoning by dimethoxy compounds as a group,[87] and several reports[7,8,53,77,105] indicate lack of efficacy. However, oxime treatment of patients poisoned by dimethoxy compounds has been beneficial in many cases.[3,12,13,79,87] Thus, 2-PAM in particular and oximes in general are recommended for therapeutic use in severe poisoning by either diethoxy or dimethoxy phosphorus compounds.[47]

Two factors contribute to the safety of oximes in the treatment of acute poisoning by organophosphorus compounds: recommended doses are small, compared with doses likely to cause even mild toxic effects in normal subjects; and adverse effects of oximes are reduced in the presence of poisoning by an organophosphorus compound.

More reports deal with 2-PAM than with other oximes in the treatment of poisoned patients. High dosages have been used in patients who survived without sequelae. Milthers *et al.*[77] reported the administration of 21 g. Gitelson *et al.*[39] administered total doses as high as 24 g in 6 d. Namba[81] reported the use of 40.5 g in 7 d, with 26 g in the first 54 h. Hiraki *et al.*[49] administered 65 g in 16 d. Warriner *et al.*[110] gave 92 g in 23 d; this case is especially instructive, because the patient suffered a relapse on the tenth day of illness after his dosage was reduced, but responded favorably to a temporary return to full dosage (12 g/d).

In one case of successful treatment with toxogonin, 16 doses of 250 mg each (total, 4 g) were administered during the first day of illness and somewhat smaller doses later.[5]

DELAYED AND LONG-TERM EFFECTS

Appendix B reviews some important animal studies of cholinesterase reactivator chemicals. The extensive literature reviewed offers little definitive information with which to project possible long-term effects or delayed sequelae in human subjects tested at Edgewood. These compounds have a short biologic half-life of 1 to 3 h. However, no chronic studies were found. Consequently, the carcinogenic potential of cholinesterase reactivators remains unknown.[61,96,98,99] Acute effects have been reported that may be reversible, but these require additional study. Reports in the Polish literature (see Appendix B) that single exposure to obidoxime (toxogonin) may affect the integrity of renal tubules need exploration and confirmation. These findings certainly raise questions about the long-term use of these compounds in humans. Another finding that needs further investigation, substantiation, and extension is the apparent production of muscle necrosis from intramuscular injection of 2-PAM[1,95] and P2S.[1] It is not known to what extent and under what circumstances this effect occurs in humans. Oral administration of cholinesterase reactivators for up to 17 wk produced erosion of the mucosa along ridges of the rugae in the fundus of the stomach and fibrosis in vicinities of the cardiac and pyloric sphincters.[1] The finding that an azotemic subject had markedly decreased clearance from blood[56] is of interest, although probably not pertinent to the Edgewood studies on human subjects, because the volunteers were in good physical condition when tested. These findings require further analysis to determine dose-frequency and dose-response relationships and their potential significance with regard to human experiments conducted at Edgewood.

EFFECTS ON VOLUNTEERS

The medical records of the volunteers who received cholinesterase-reactivating chemicals consisted of the test protocol, physicians' orders, nursing notes (including clinical observations), a checklist of symptoms, and laboratory and performance test results. The reports of physicians' examinations and physical findings were generally not included. Volunteers were identified by number. The Committee on Toxicology's assessment was based on records and summaries provided by the Department of the Army and NRC staff. The procedures were described fully in Volume 1. In most cases, the analysis was based on summaries of drug administrations prepared by a consultant to the Panel.

The number of subjects tested with each compound, the number of records examined, routes of administration, and doses are shown in Tables 2-8 through 2-11. In some instances, the compounds were given before or after anticholinesterase compounds or in conjunction with other drugs; thus, some of the results shown here were also presented in Volume 1.

The data on TMB-4 (Table 2-11) are difficult to interpret, because, in the instances on which clinical data are available, other potent drugs were given as well.

The manifestations experienced by the subjects in these tests (Tables 2-8 through 2-11) were the moderate clinical effects that

TABLE 2-8

Manifestations after Administration of 1-7 Doses (on Different Days) of 2-PAM (Chloride Form) to 79 Subjects[a]

Manifestations[b]	Intravenous, 5 mg/kg, to total of 500 mg, N = 29	Oral, 5-7 g total, N = 13	Intramuscular, 2.5 mg/kg, to total of 600 mg, N = 37[c]
None	0	6	0
Dry mouth[b]	2	--	--
Dizziness	23	1	--
Diplopia	8	--	--
Eye discomfort	12	--	--
Blurred vision	9	1	5
Mood elevation	1	--	--
Voiding difficulty	2	--	--
Nausea	6	--	1
Vomiting	1	--	--
Uric acid increase	1	--	--
Faintness	1	--	--
Claustrophobia	1	--	--
Abdominal cramps	1	--	--
Diarrhea	--	3	--
Muscle pain	--	--	37
Tachycardia[b]	--	--	1
Grand mal seizure[d]	--	--	1

---

[a] Task plan: absorption efficacy of 2-PAM (chloride form) as function of pH (intramuscular studies). Studies conducted: plasma and urine content, renal clearance, CNS effects, blood pressure, and in some cases, pupil size and number facility. Test conditions: heat and exercise in some tests. Clinical evaluation tests: uric acid, creatinine, BUN, SGOT, and CPK in serum. Records on 79 subjects selected, on basis of high dose or high frequency of administration, from records on 607 subjects tested.

[b] Dry mouth and increased heart rate were associated with atropine administrated to some subjects.

[c] Several subjects received atropine in addition to 2-PAM.

[d] Subject 6849 (see text).

TABLE 2-9

Manifestations after Administration of Toxogonin to 41 Subjects[a]

Manifestations	Intravenous,[b] 0.5-1.0 µg/kg, 60-128 mg infused, N = 11	Oral, 1-9 g total, N = 20	Intramuscular, 2.5-10 mg/kg, N = 10
None	0	3	0
Hot, cold, numb, tingling sensation	10	9	10
Eye discomfort	1	2	2
Dizziness	1	2	1
Dry mouth	--	--	2
Local pain	--	--	10
Peculiar taste	--	10	1
Drowsiness	1	5	--
Blurred vision	1	1	--
Headache	--	3	--
Nausea	--	3	--
Vomiting	--	1	--

---

[a] Records on all 41 subjects tested were summarized.

[b] Five subjects, single injection; six subjects, infusion as total dose in combination with aminohippuric acid (PAH).

TABLE 2-10

Manifestations after Administration of P2S to 75 Subjects[a]

Manifestations	Intra-venous, 5 mg/kg, N = 3	Oral, 2-9 g total, N = 49	Oral Before Nerve Agent, 1-3 g total, N = 12	Intramuscular After Nerve Agent, 1-2 g total, N =11
None	3	38	11	10
Headache (slight)	--	2	--	--
Voiding difficulty	--	4	--	--
Diarrhea	--	4	--	--
Stomach ache	--	2	--	--
Nausea	--	1	--	--
Ventricular extrasystoles	--	--	--	1
Anxiety, agitation, vomiting	--	--	1[b]	--

---

[a] Clinical tests included CBC, blood oximes, creatine, and urinalysis. Results were in normal range after transitory manifestations. Nerve agent resulted in decrease in RBC cholinesterase content. Records on 75 of 95 subjects tested were summarized; each subject was tested once with P2S.

[b] Subject 2307 (see text).

TABLE 2-11

Manifestations after Administration of TMB-4 to 24 Subjects[a]

Protocol	No. Subjects
TMB-4 alone	6[b]
With intravenous soman	1
With intravenous soman and P2S	1[c,d]
With percutaneous VX and atropine	2
With percutaneous VX, atropine, and 2-PAM (chloride)	1
TAB[e]	3[d]
TAB and heat stress	2[d]
TAB and physostigmine	4

Manifestations[f]	No. Subjects
None	--
Dry mouth	9
Blurred vision	6
Lethargy, sluggishness	6
Dizziness	4
Dreaming (hallucinations)	3
Nausea	2
Restlessness	2
Muscle twitching	2
Heat, flush	1
Headache	1
Abdominal discomfort	1
Anxiety, agitation, vomiting	1 [c]

---

[a] Records on 24 of 34 subjects tested were summarized; clinical data available on 11, only laboratory data on eight, and no data (except RBC cholinesterase content) on five.

[b] Clinical data available on one.

[c] Subject 2307; see text.

[d] Clinical data available.

[e] TAB = TMB-4, atropine, and benzactyzine.

[f] Among 11 with clinical data.

have been reported in the literature, and they are discussed in Appendix B. In all but two instances (described below), moderate effects disappeared within 24 h.

One volunteer (2307) received 2 g of P2S and 1 g of TMB-4 orally 68 min before being given 1.5 µg of soman by intravenous infusion. He had minor symptoms, but in 12 h became anxious, restless, and agitated. He was transferred to Walter Reed Hospital. His Multiphasic Multiple Personality Inventory (MMPI) and psychiatric interview before exposure were described as "doubtful in regard to suitability, but not grossly pathological." Postexposure physical and neurologic examinations showed no organic signs of neurologic disease. The discharge diagnosis from Walter Reed Hospital in 1961 specified acute, moderate anxiety reaction manifested by restlessness, anxiety, agitation, and hysterical reaction. He has experienced further problems requiring inpatient and outpatient psychiatric care. The Veterans' Administration diagnoses between 1966 and 1980 noted infantile personality with strong paranoid trends, organic brain syndrome, and severe anxiety neurosis with depression. This subject was also noted in Volume 1 (p. 30).

A second subject (6849) experienced a grand mal seizure 3 h after receiving 300 mg of 2-PAM (chloride form) intramuscularly. He regained consciousness within 5 min; he had bitten his tongue. He was initially lethargic, but felt well 10 h later. He was transferred to Walter Reed Hospital, but followup records are not available. He had received 300 mg of 2-PAM intramuscularly 5 and 8 d before the episode. The only symptom on those occasions was local pain at the injection site.

Although 2-PAM is an FDA-approved drug, data submitted to the FDA to obtain approval are proprietary and were not released when requests for them were made to the FDA and to the manufacturer. A report of the FDA Adverse Drug Experiences Monitoring Program (October 30, 1983) contained one adverse reaction (hypotension following 1 g by intravenous injection).

In summary, with the possible exception of those two cases, the records contained no evidence of delayed or persistent effects after administration of the cholinesterase reactivators. Such data cannot, however, address the issue of long-term effects or delayed sequelae.

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CONCLUSIONS

On the basis of an examination of toxicologic literature, case reports from Edgewood volunteers, and a review of mortality data (reported in Volume 1), the Committee found no evidence of chronic disease in animals or humans associated with single or repeated doses of the cholinesterase reactivators (oximes). The paucity of chronic-exposure data from animals and the lack of followup data on volunteers prevent certainty in predicting occurrence or absence of delayed effects. The compounds are eliminated very rapidly from the body, but they produce a variety of acute effects that are short-lived and reversible, such as gastrointestinal distress following oral administration, pain at an injection site, dizziness, headache, and ocular discomfort. The Committee found no data on the basis of which to determine or rule out carcinogenicity, mutagenicity, teratogenicity, or reproductive effects of the four oximes and therefore did not reach a conclusion in this area.

3

PSYCHOCHEMICALS

BACKGROUND

Military interest in psychochemicals stems from the late 1940s. L. Wilson Green of the Chemical Corps Technical Command at Edgewood proposed that modern military use of psychochemicals might permit the conquering of an enemy without the need for weapons of mass destruction. Such use, he suggested, might reduce the wholesale killing, human misery, and property destruction normally experienced in warfare. He proposed a search for a stable chemical with the capacity to produce mental abnormalities of military importance; 61 chemicals were suggested as a starting point for this search.[1]

Over the next few decades, scientists at Edgewood engaged in toxicologic and clinical evaluations of the biologic effects of a wide variety of chemicals that could alter the state of mind or mood, largely by affecting the brain. Among those tested on human volunteers were LSD, a hallucinogen; BZ (3-quinuclidinyl benzilate) and related anticholinergic compounds; phencyclidine, an anesthetic with marked disorienting after-effects; and dibenzopyrans, CNS depressants with powerful capacity to produce orthostatic hypotension.

LSD and the anticholinergics were the subjects of earlier extensive evaluations.[2,3] This chapter is concerned only with phencyclidine and dimethylheptylpyran (dibenzopyran) and its isomers.

Table 3-1 lists the compounds tested, approximate numbers of subjects, routes of administration, and dosages.

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VOLUNTEER SCREENING, SELECTION, AND CLASSIFICATION

In the clinical charts at Edgewood, severe adverse psychologic reactions to SNA or to the cannabinoids appeared less common than one might expect from experience in civilian laboratories. If these findings were valid, they might be accounted for by subject selection procedures and special characteristics of the experimental milieu.

Studies of workers often find that workers are healthier than the general population. This "healthy-worker effect" is generally ascribed to the fact that the working population is in better overall health than the general population, which includes the very sick, the elderly, and the hypersusceptible.

The selection of volunteers for testing at Edgewood most likely introduced a "healthy-test-subject effect" into the study. In fact, those selected for exposure to the test chemicals were healthier than those used as controls or used in nondrug tests of equipment. The control groups consisted of those who were rejected for drug testing and thus possibly less healthy. Because the exposed subjects were healthier at the start than the nonexposed subjects, comparisons between these two groups may well yield results that understate the relative risk to the exposed subjects. For the study of neurologic processes and psychologic functioning, subtle effects in the exposed subjects would not be readily evident in a comparison of them with the less healthy, nonexposed subjects.

In addition, there was a great deal of preselection, in that all the subjects were soldiers--healthy enough and functioning well enough to meet the criteria for entry into the Army. A detailed set of guidelines, most completely spelled out in a document dated August 12, 1968, described a standard operating procedure in the clinical research department (Appendix A, part 2) for forming the exposure group. Multiple criteria were used in the psychologic screening of volunteers. A "yes" answer on any of various items in the medical history without explanations based on further examination by a medical officer would routinely be cause for rejection. When General Test (GT) scores were in the very low range (below 90 or 80), the volunteer was rejected.

Minnesota Multiphasic Personality Inventory (MMPI) profiles were used as approximate guidelines or rules of thumb, rather than to pro-

TABLE 3-1

Psychochemicals Tested on Human Volunteers at Edgewood

Military Designation	Tox. No.	Chemical Type	Common Name	Abbreviation	CAS No.	No. Subjects Tested	Dosage, µg/kg	Route[a]
EA 1476	H-1	Dibenzopyran	Dimethylheptyl pyran	DMHP	32904-22-6	38	0.4-4,000	PO
EA 2233	H-2	Dibenzopyran	Dimethylheptyl pyran	DMHP	39624-99-2	95	0.5-5.4 0.5-8.3 2.5-51.4	IM IV PO
EA 2233-1	H-3	Dibenzopyran	Dimethylheptyl pyran	DMHP	14950-75-5	23	1-10.5	IM
EA 2233-2	H-4	Dibenzopyran	Dimethylheptyl pyran	DMHP	14950-76-6	15	0.5-7.5	IV
A 2233-3	H-5	Dibenzopyran	Dimethylheptyl pyran	DMHP	15077-23-3	20	1.0-10.5	IM
EA 2233-4	H-6	Dibenzopyran	Dimethylheptyl pyran	DMHP	14950-77-7	16	0.7-7.8	IV
EA 2233-5	H-7	Dibenzopyran	Dimethylheptyl pyran	DMHP	14950-78-8	6	0.7-3.5	IM
EA 2233-6	H-8	Dibenzopyran	Dimethylheptyl pyran	DMHP	14950-79-9	6	0.7-6.4	IM
EA 2233-7	H-9	Dibenzopyran	Dimethylheptyl pyran	DMHP	15206-43-6	4	5.3-6.4	IM
EA 2233-8	H-10	Dibenzopyran	Dimethylheptyl pyran	DMHP	14950-80-2	6	0.7-3.5	IM
EA 2233-24	H-11	Dibenzopyran	Dimethylheptyl pyran	DMHP	39624-99-2	30	1.2-2.5	IV
EA 2148-A	C-1	Heterocyclic amine	Phencyclidine	SNA	956-90-1	29	2.5-43	PO[b]

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[a] PO = oral; IM = intramuscular; IV = intravenous

[b] Some subjects exposed to aerosol at 250-888 mg/m^3; Cts 249-2,955 mg·min/m^3.

vide firm cutoff points. In general, any prospective subject who had five or more clinical scales on the MMPI above 65 units was not included in the experiments. A volunteer with high L and K scales was considered sutiable for the experiments only after careful review of family history and of other indication of possible psychologic problems. Any subject with high PD, PA, and SC scales (psychoticism) was rejected, as was a subject with high PD, MF, and MA patterns (sociopathic deviation), particularly if there was a history of "acting out." Careful review of the overall clinical picture, history, etc., took place if the HS, D, HY, PT, and SI patterns (neuroticism) were high. Apparently, exceptions were made--for example, if an ambitious college graduate had high PD and MA scales, but no history of acting out. In doubtful cases, corroborating evidence from the family history was reviewed. Particular attention was paid to the family and developmental histories of prospective volunteers, especially a history of trouble in school, contact with a psychiatrist for anything other than routine screening, fighting in association with drinking, and overt expressions of hostility.

Screening of the histories and MMPI profiles took place before arrival at Edgewood. After arrival, volunteers were interviewed by officers in the Psychopharmacology Branch. On the basis of further testing (Sentence Completion and Picture Frustration tests), physical examination, and interview, subjects were classified on a four-point scale. Those rated A were considered suitable for psychochemical testing; those rated B were suitable for low-dose psychochemicals only; those rated C were not suitable for psychochemicals; those rated D were suitable for equipment testing only. The main criteria for an A or B rating were absence of evidence of psychologic problems, absence of a tendency to somaticize or act out intrapsychic tension, good ego strength, flexibility, maturity, good sense of identity, normal MMPI, and family history. Subjects who seemed to be particularly at ease when handling anxiety and hostile or aggressive impulses were rated A+--suitable for psychochemical tests considered to be of greater than usual stress. Those rated B were similar to the A group, but had had occasional experiences that suggested less control or minor personality disturbances. Any subject who showed a tendency toward psychosomatic reaction or aggressive acting out, who appeared to be dull or nonverbal, or who had obvious neurotic traits, immaturity, or rigidity was not included in any psychochemical experiments.

The criteria described in the standard operating procedure appear to be those used either consciously or deliberately in many civilian laboratories that conduct research with psychoactive drugs with normal volunteers. It appears that the subjects actually given psychochemicals in these experiments were selected from an optimal pool of mentally and physically healthy persons.

Apparently, the precise configuration and staffing of the research unit changed over the years during which these experiments occurred. Facilities for the subjects included a controlled environment with padded rooms (to protect subjects from harm from hypotension) and an adjacent communal area. During the first hours of the experiment, each subject was in his own padded area with a padded stool. Each subject had a nurse or aid in the room during his progress. Later, the subjects were allowed access to dayroom facilities, where they played cards. When further along in recovery, they could play table tennis. Throughout the entire recovery process, they could be observed through a window.

The notes, comments, and data in the charts reflected what must have been a supportive and well-staffed research unit. The only negative comments from subjects in the charts had to do with the quality or preparation of the food. Many positive comments reflecting careful attention, support, and, in general, informed participation in the experiments occurred throughout the charts over the years. Retropective chart reviews always involve guessing and speculation. A best guess is that a supportive atmosphere and careully screened and well-informed (with respect to experimental procedures, goals, pitfalls, etc.) subjects were important in determining how well these volunteer subjects tolerated the experiments.

It is difficult to generalize about the experimental design used in studies of multiple drugs and spanning 10 yr or more. Some generalizations are relevant in assessing the quality of data, pattern of effects, and possible consequences. In the usual sequence of experiments, the effects of low doses were investigated first in a few volunteers, particularly when the route of administration was being changed or when a compound that had been studied previously only in animals (such as the cannabinoids) was being studied. Then, depending on the pattern and duration of effects, a small to moderate-sized group of subjects were tested with a few doses in what appeared to be a safe but pharmacologically active range. Later studies followed up on interesting or possibly worrisome side effects, such as borderline changes in hepatic or renal function. Particularly in later studies based on earlier observations, interventions were made with assumed antidotes or antagonists; for example, drugs that increased blood pressure were given in conjunction with or after dimethylheptylpyran to investigate the intriguing and important postural hypotension.

The protocols appeared to be flexible and generally conservative, with variations often following up preliminary observations. A critical and skeptical reviewer, in retrospect, might say that there was too great emphasis on browsing and that the changes in protocol, with small groups tested under any single protocol, precluded definitive conclusions. These experiments were analogous to the Phase 1 clinical trials in human beings now conducted with therapeutic drugs, in which examination of small groups, attention to borderline biochemical abnormalities, revision of protocols, and attention to hunches about mechanisms of drug action, dose, etc., are appropriate research strategies.

Placebo controls were not used and were probably not appropriate, given the goals of the research. One must remember that when these studies began, in the early 1960s, psychopharmacology, particularly optimal research strategy and design as we know them today, was truly in its infancy. Not until the mid-1960s was there a general consensus in a minimally acceptable design for studying psychochemicals, and even now there may be disagreement. The experimental design used in the experiments at Edgewood compares favorably with the pharmacologic research at other research centers.

In the Edgewood studies, SNA was administered intravenously, orally, and by inhalation.

Intravenous doses of 0.1 mg/kg given to 10 volunteers produced onset of physiologic and psychologic effects within 3-5 min of injection that peaked about 10 min to 1 h after injection. Most of the symptoms were nonmeasurable 5-6 h later. As in all studies with SNA, individual variation was great. For example, of the 10 volunteers given intravenous SNA, four became withdrawn and drowsy and answered when spoken to, but otherwise were silent. The effects on proprioception were manifested by limb numbness, vertigo, ataxia, and a feeling of detachment. Various degrees of amnesia regarding the events after the first hour after injection were common. Nausea was common, but considered to be less than expected by the investigators, possibly because the subjects were nonambulatory. Increases in systolic and diastolic blood pressure of up to 20 or [illegible] Hg occurred soon after injection and lasted for 2-3 h. It was judged by subjects and observers alike that SNA would impair performance in a military field situation.

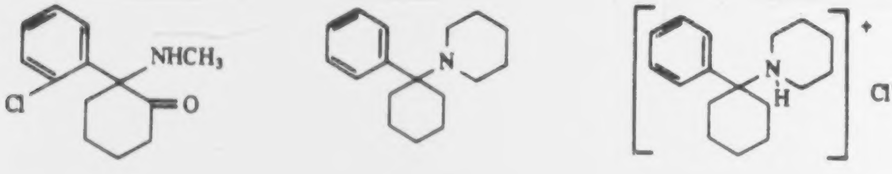
After oral doses of up to 30 mg (0.48 mg/kg), similar signs and symptoms appeared in a single test subject. At 30 mg, approximately the first plane of stage three anesthesia was produced. The one subject used was unresponsive to sensory stimuli, although corneal reflexes were intact. Blood pressure was slightly increased. An hour after the drug administration, he was responsive to stimuli, but still groggy; 8 h later, he had recovered almost totally, but was amnesic regarding the events of the hours after the drug was administered. Two volunteers given a combination of SNA (20 mg) and alcohol had intense "manic" reactions, with much agitation and restlessness. Perceptual and cognitive effects lasted 4 d in one and 2 d in the other. At lower oral doses (5 and 10 mg), volunteers described their subjective feelings as similar to those of alcohol intoxication. Skin temperature and heart rate increased slightly. Vertigo, ataxia, weakness, and nausea were mild and not sufficient to interfere with treadmill exercises that were part of the experiment. Subjects appeared slow to think and slow to make decisions and reported a subjective slowing down of time. The subjective effects peaked at about 2-4 h after oral administration and, after the 10-mg dose, were still present to a slight degree at 13-14 h. After the 5-mg dose, effects were generally milder, and subjective symptoms were gone in about 8 h. Perceptual motor functioning, as judged by Purdue pegboard and Minnesota rate manipulation tests, was definitely impaired after the 10-mg oral dose, markedly impaired after 15 mg, and nearly absent after 5 mg.

REVIEW OF AVAILABLE INFORMATION ON PHENCYCLIDINE

CHEMISTRY

Phencyclidine (Sernyl, SNA)--1-(1-phenylcyclohexyl)piperidine, $C_{17}H_{25}N$ (molecular weight, 243.38)--is an arylcyclohexylamine. It is used as the hydrochloride, $C_{17}H_{25}N\ HCl$ (molecular weight, 279.84), which is crystalline with a melting point of 214-218°C. Its hydrobromide salt is also crystalline with a melting point of 214-218°C. The hydrochloride is soluble in water, methanol, ethanol, aniline, and methylene chloride, and the base is almost insoluble in water and soluble in toluene, methanol, ethyl acetate, kerosene, and methylene chloride. The base (SNB) is crystalline with a melting point of 46-46.5°C.

The chemical structures of SNA, ketamine, and SNB are shown in Figure 3-1.



Ketamine

base (SNB)

hydrochloride (SNA)

FIGURE 3-1 Structure of ketamine, SNA, and SNB.

SNA was originally synthesized and developed as an anesthetic agent for human use by Parke, Davis and Co. under the name Sernyl.[13-15] Its human use was soon abandoned, because it sometimes produced postoperative thought disturbances and agitation. It is currently used, under the name Sernylan, as an immobilizing agent in veterinary medicine.

In pharmaceutically pure form, SNA is a white powder that dissolves readily in water. When distributed illicitly, phencyclidine, often called PCP, is highly variable in appearance (powder or tablets in many colors or as liquid), contains many impurities, is often adulterated, and quite often is misrepresented as another drug, such as marijuana constituents, mescaline, psilocybin, lysergic acid diethylamide (LSD), or even amphetamine or cocaine.

The compound tested at Edgewood was pure phencyclidine provided by a pharmaceutical manufacturer and should not be confused with the substance sold on the street as PCP.

The structure of SNA was modified by replacement of its phenyl, cyclohexyl, and piperidine rings and by introduction of substituents onto those rings. Replacement of the phenyl ring with a thienyl ring increased central activity, but bulkier aromatic rings were inactive.[39] Replacement of the piperidine ring by $NHCH_3$ and some substitutions in the other rings led to the development of ketamine, an effective dissociative anesthetic agent.

ABSORPTION, FATE, AND ELIMINATION

Two recent reviews[3,22] contain considerable information on the absorption, distribution, metabolism, and excretion of phencyclidine.

Absorption

Early studies in experimental animals and human subjects[46] established that SNA is well absorbed when administered by inhalation, percutaneously, intraocularly, orally, intramuscularly, intravenously, and intraperitoneally. Dogs exposed to aerosols generated from a 15% aqueous solution of SNA developed prostration, hypersalivation, and exophthalmos; approximately 50% of the dogs had tremors and convulsions. SNA and SNB were dissolved in a number of vehicles, and these preparations were applied to the skin of rabbits. Ataxia confirmed the percutaneous penetration of the drug. This clinical sign also occurred after an alcoholic solution of the base or the salt was instilled in the eyes of rabbits. A human volunteer given SNA orally at 0.48 mg/kg reached plane 1 of stage 3 anesthesia 1 h after administration. Intravenous administration of SNA at 0.01 mg/kg in 10 male volunteers resulted in onset of mental and physical effects within 3-4 min. Nineteen men were given aerosolized SNA in methylene chloride solution. At an exposure as low as 250 $mg \cdot min/m^3$, one subject developed visual disturbances and lightheadedness in 7 min and perioral and distal paresthesias within 10 min. Inadvertent exposures by inhalation have been reported. Aniline _et al._[4] found SNB in the blood of a 65-yr-old woman who occupied a second-floor apartment directly above a clandestine laboratory making SNB by an open-vat process. Pitts _et al._[52] reported intoxication in two chemists, employed in a law enforcement laboratory, who handled confiscated samples of SNA.

Absorption through the placenta has been shown in mice and rabbits[50] and is likely to occur in humans.[30] In the mouse, SNA concentration was 10 times higher in fetal tissue than in maternal blood. Furthermore, SNB appeared rapidly in the milk, reaching concentrations 10 times those in maternal plasma. Fetal SNA concentrations in the rabbit peaked 12 h after parenteral administration to the dam. A neonatal infant whose mother had used SNA during pregnancy manifested abnormal behavior consistent with effects often seen with this drug. However, blood SNB concentrations were not measured in either child or mother. Aniline and Pitts studied three women who used SNA during pregnancy;[3] blood and urine SNB concentrations were determined. Concentrations in cord blood were 2-3 times higher than those in the mother in all cases.

Distribution

In Sprague-Dawley rats given SNA at 50 mg/kg intraperitoneally, concentrations in adipose tissue 1 h after injection were 13 times higher than those in brain and more than 20 times higher than those in blood.[34] Thus, SNB is highly lipophilic in its distribution; indeed, SNB remained in fat at approximately 10 μg/g 48 h after injection--that is equivalent to the highest blood concentration attained (3 h after injection). Brain and blood concentrations were virtually parallel. In one case of an SNB-related death in a human subject,[6] the brain:blood ratio of SNB concentration was 6:1, and the liver:blood ratio was 2:1. In a second case in which it was established that the deceased had smoked SNB, concentration ratios were as follows: liver:blood, 46:1; lung:blood, 2:1; and kidney: blood, 1:1. In a third case, in which intravenous use of SNB was documented, the liver:blood and bile:blood ratios were 4:1 and 1:1, respectively. A knee synovial fluid:plasma ratio of 9:1 was found in a living subject who had inhaled ("snorted") SNB.

Receptor sites specific for binding SNB have been found in rat brain and other organs.[66] The greatest specific binding was found in the cerbral cortex and the corpus striatum. Binding was less in the thalamus and hippocampus and least in the medulla oblongata, pons, olfactory bulb, hypothalamus, and cerebellum; none was found in the spinal cord. Specific binding was also found in heart, liver, lung, and kidney.

Distribution studies of [^{3}H]SNB in selected brain regions[68] revealed concentrations from 10.4 nmol/g in cerebellum to 16.4 nmol/g in anterior cingulate cortex. Another study[37] showed that radioactivity from administration of [^{3}H]SNA was distributed almost evenly over the major anatomic areas of the brain; only the hypothalamus had a high concentration. Most of the radioactivity was associated with the soluble cell fraction, and very little was detected in nuclear and mitochondrial fractions. Chronic administration of SNA altered the distribution of [^{3}H]SNB and its metabolites in the central nervous system; radioactivity in the cortex was 7-31% less than that in the whole brain. Other areas, particularly the hypothalamus, had a higher concentration relative to that in whole brain. A recent report[44] indicated that 30 min after intravenous or oral administration of [^{3}H]SNA to mice the highest concentration was in the stomach. The next highest concentrations were in fat (by the intravenous route) and in liver and intestine (oral route) and the lowest in brain and plasma (either route).

Metabolism

A proposed scheme of the metabolism of SNB in man was reported by Jasinski *et al.*[35]

After mice became tolerant to SNB, hepatic microsomal cytochrome P-450, cytochrome b5, nicotinamide adenine dinucleotide phosphatase, and NADPH-cytochrome c reductase activities were increased and thus presumably involved in SNB metabolism.[49] These findings confirmed previous observations that chronic SNB administration to the mouse increased liver microsomal hydroxylation of aniline, pentobarbital, and hexobarbital and the *N*-demethylation of aminopyrine and ethylmorphine.[32] 4-Phenyl-4-piperidinocyclohexanol (PPC), one of the major metabolites of SNB, can exist in *cis* and *trans* isomers. Both isomers were found to be biologically active in the mouse, producing ataxia and seizure activity (the latter at high doses). The *trans* isomer was only slightly more active than the *cis* isomer.[11] The presence of an additional metabolite in the urine of human subjects using SNB has recently been confirmed;[16] the metabolite had been found in rat and rabbit liver and in dog urine. This metabolite was identified as 5-(1-phenylcyclohexylamino)valeric acid. In man, PPC is highly conjugated as the glucuronide and is excreted mainly in that form.[35] Details of the biotransformation of SNB have been extensively reviewed.[40]

Elimination (Pharmacokinetics)

It was recognized many years ago that SNB had a prolonged action.[46] A human volunteer given SNB orally at 0.48 mg/kg was not responsive to stimuli until 4 h after treatment, at which time he was stuporous, and his arms and legs remained in any position in which they were placed. Full recovery required about 7 h. In Sprague-Dawley rats, half-lives of SNB were approximately 1 h in blood, 2 h in brain, and 3-4 h in adipose tissue.[34] Plasma half-lives of 2.4 h (monkey) and 2.9 h (dog) have been reported.[69] The half-life in humans varies considerably from subject to subject. Acidification of urine results in a markedly decreased half-life.[24] Some indication of the slow elimination of SNB is found in an investigation of coroners' cases of persons who died of accidental causes;[6] the highest concentrations of SNB in brain and other organs were noted 7-10 d after single high doses of the drug. In brains of SNB-tolerant mice, the half-life was much shorter than that in control animals.[49] SNB metabolites remained in the liver of mice up to 14 d and in the lung up to 21 d.[44] Measurable concentrations of SNB persisted in a human subject for at least 6 mo after the last known exposure.[52] All available information indicates that the pharmacokinetics of SNB are highly dose-related.

The recently reported findings of Cook et al.[19] on the biodisposition of phencyclidine after oral or intravenous administration of trace amounts of radiolabeled material are presented in Table 3-2.

Quantitatively similar dispositional kinetics in the dog were recently published;[71] again, there was a wide variability in half-life, as well as a very small renal clearance.

These results indicate that, in addition to effectiveness by inhalation and parenteral administration, SNA is well absorbed in man when administered orally. The drug is 60-70% bound to plasma proteins, the volume of distribution is high (approximately 500 L in an 80-kg man), clearance is largely a result of metabolic processes, the half-life is quite variable from one person to another, and the drug and its metabolites are excreted principally in the urine, regardless of whether it is given orally or intravenously.

ANIMAL TOXICOLOGY

Several animal species (mouse, goat, cat, dog, guinea pig, rabbit, and rat) were given graded doses of SNA intravenously to determine its LD_{50} and its median effective dose (ED_{50}) for several gross manifestations--ataxia, salivation, prostration, and convulsions. Table 3-3 lists the LD_{50}s and ED_{50}s for these effects. It shows that in various species the LD_{50}s ranged from 11.7 to 17.9 mg/kg, and the pharmacologic dose ranged from 50 to 80 μg/kg; thus, the margin of safety of this compound was high. Ataxia, salivation, and prostration occurred within 1-5 min, and convulsions developed within 1-40 min. Death usually occurred in less than 15 min in the guinea pig, rabbit, and cat; in 1-3 h in the mouse; and in 5 min to 24 h in the dog. At 3-5 mg/kg intravenously, SNA caused a decrease in blood pressure, a decrease in heart rate, and minor cardiac irregularities in cats. It also significantly depressed respiration, but

TABLE 3-2

Biodisposition of Phencyclidine in Man[a]

Disposition of trace amount of phencyclidine:	
Oral bioavailability	72 ± 8%
Plasma concentrations (1 h after 1-mg intravenous dose)	10 pmol/ml
Plasma binding	68-69%
Volume of distribution	6.2 ± 0.3 L/kg
Total clearance	380 ± 80 ml/min
Renal clearance	33 ± 8 ml/min
Apparent terminal half-life	17.6 h (7-50 h)
Renal excretion (10-d collection)	73 ± 4%
Fecal excretion (10-d collection)	5 ± 1%
Metabolites of phencyclidine in urine (97% of total ^{3}H label):	
Phencyclidine (SNB)	16%
1-Phenylcyclohexylamine (PCA)	trace
1-(1-Phenylcyclohexyl)-4-hydroxypiperidine (PCHP)	7%
4-Phenyl-4-piperidinocyclohexanol (PPC)	18%
4-(4'-Hydroxypiperidino)-4-phenylcyclohexanol (dihydroxy metabolite)	3%
5-(1-Phenylcyclohexylamino)valeric acid	15%
Unidentified polar metabolites	38%

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[a] Data from Cook *et al.*[19]

TABLE 3-3

Lethal and Effective Intravenous Doses of SNA in Various Species[a]

Species	ED$_{50}$, mg/kg[b]							Highest No-Effect Dose, mg/kg[b]
	Salivation	Ataxia	Prostration[c]	Convulsions[c]	LD$_1$, mg/kg[b]	LD$_{50}$, mg/kg		
Mouse	NR	NR	0.3	NR	Ca. 11.3	16.0[d] (14.4-18.8)		
Goat	0.5	0.025	0.1-0.5 (6 h)	0.1-0.5	Ca. 8.9	12.0 (10.9-13.2)		<0.025
Cat	<0.5	0.025-0.05	5.0-7.9	Irregular at 7.9 and above (6 h)	Ca. 12 (3 d)	Ca. 15		0.025
Dog	0.5	0.05-0.1	0.1-0.5	0.75 (1.2 h)	Ca. 14.6 (2-6 h)	17.9 (16.7-19.1)		0.05
Guinea pig	NR	<0.25	0.25-0.5 (15-30 min)	5.0 (20-60 min)	Ca. 7.1	12.0 (10.0-14.4)		<0.25
Rabbit	NR	0.20-0.79	0.5-4.0 (5-15 min)	Irregular at 7.9 and above (3 h)	Ca. 9.4	11.7 (10.9-12.6)		0.05
Rat	NR	0.4	NR	NR	NR	NR		0.25

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[a] Data from McNamara *et al*.[46]

[b] NR = not recorded

[c] Times in parentheses are durations of action or times of effect.

[d] Intraperitoneal LD$_{50}$ in mice was 66±5 mg/kg; most of the mice died within 10 min of injection.

had no neuromuscular blocking effect; that indicates that its respiratory depressant effect is not central.[46,62]

Death from SNA intoxication appears to involve both cardiovascular and respiratory systems.[46,62] SNA increases the effects of pentobarbital on both respiration and heart rate. The depressant effects of SNA on the myocardium and on the Purkinje system are antagonized effectively by epinephrine, but not by Metrazol (pentylenetetrazol) and caffeine. Artificial respiration restored cardiac stability and regularity. It was suggested that SNA interferes with oxygen utilization in animals. The circulatory and respiratory responses to SNA were considered to be mediated through the medulla oblongata.[46,62]

The primary action of SNA is on the brain.[3] In low doses, it produces tranquilization in the pigeon, cat, dog, guinea pig, and monkey. In high doses, it causes repetitive convulsions. The inhalation toxicity of SNA and its base (SNB) given as aerosols was studied in mice. The LCt_{50} for SNA was 22,000 mg·min/m^3. Mice and possibly dogs were more susceptible than other species to the lethal effects of aerosols of SNA. The LCt_{50}s for rats and guinea pigs were in excess of 115,000 mg·min/m^3.[46] Recent data of Davis et al. show that a presursor in synthesis of SNA, piperidinocyclohexanecarbonitrile (PCC), increases the toxicity of SNA.[21]

Early studies (1957) by Parke, Davis in monkeys showed no evidence of tolerance or persistent toxic effects in monkeys after repeated parenteral administration at 1.25 or 5 mg/kg once a day, 5 d/wk, for 43 doses.

No persistent toxic signs were seen in dogs after intramuscular injections at 1 mg/kg. Hematologic and biochemical values in the animals remained normal.

More recent studies,[8,9,12] at dosages that produce behavioral changes, have shown that chronic intramuscular or intraperitoneal administration of SNA results in development of tolerance to several test situations in several animal species. Tolerance to roughly 2-4 times as much SNA as initially given was evident in the behavioral effects of SNA in these species. Tolerance to SNA was also observed in intramuscular SNA self-administration studies in the monkey. In the development of tolerance, such pharmacologic factors as dose per injection, injection frequency, and duration of chronic exposure were considered to play a more important role than behavioral factors (e.g., reinforcement loss).[9]

Rhesus monkeys on chronic SNA intramuscular administration have also been shown to develop dependence. In experiments in which rhesus monkeys were given unlimited access to SNA, animals self-administered amounts sufficient to maintain continuous gross intoxication for periods of 50 d or longer. Unlike other hallucinogens, SNA is avidly self-administered by monkeys. Abrupt discontinuation after long-term intoxication produced withdrawal symptoms that could be reversed by further SNA administration. The spectrum of symptoms varied somewhat among the animals. Four hours after drug administration was stopped, SNA intoxication decreased and animals started eating and became less ataxic. At 8-12 h, they became markedly hyperresponsive with a distinctive oculomotor hyperactivity that was different from the nystagmus observed during intoxication. They usually refused preferred food. During the peak hour (at 12-16 h) of withdrawal, several of the following abstinence symptoms were manifested: piloerection, tremor, diarrhea, continuous vocalization, hyperresponsivity, oculomotor hyperactivity, priapism, bruxism, and ear and facial twitches. Abdominal contraction, emesis, and convulsions occurred in some animals. Food intake decreased by 50% during withdrawal. The symptoms gradually diminished over the next 24 h. Plasma concentrations of SNA during self-administration ranged from 105 to 208 ng/ml.

The large margin of safety for this compound when given intravenously, intragastrically, intraperitoneally, and subcutaneously is decreased when the compound is given in the aerosolized form. Tolerance and dependence have been observed after chronic administration.

NEUROPHARMACOLOGY

The neurochemical effects of phencyclidine in animals have been well reviewed.[3,22,45,51,53] A summary of these reviews and of some recent papers is presented below.

At behaviorally effective intraperitoneal doses (10 mg/kg), SNA has been reported to increase serotonin (5-HT) concentrations in rat brain.[60] 5-Hydroxyindoleacetic acid concentrations are first decreased and then increased. It has been claimed that SNA causes a decrease followed by a compensatory increase in 5-HT turnover. However, these studies have been criticized on experimental grounds,[36] and later studies showed that SNA produces a decrease in 5-HT.[58] No effects on aromatic amino acid decarboxylase and monoamine oxidase, two enzymes involved in 5-HT formation and destruction, were observed SNA has been shown to reduce the concentration of the 5-HT precursor tryptophan. The uptake of 5-HT into rat brain preparations in vitro and into the brain stem in vivo is somewhat inhibited. There also seems to be a strain difference in response to SNA.[61] In general, acute administration of SNA does not seem consistently to cause marked changes in brain serotonin content or turnover.

Similarly, the effects of SNA on the catecholamine (norepinephrine, epinephrine, dopamine) systems have been widely studied, but the results are far from clear. SNA has been reported to increase rat brain tyrosine[59] and to decrease rat brain norepinephrine (NE) and to increase dopamine (DA) slightly.[60] Again, conflicting results are available.[31] Apparently, changes in these biogenic amines depend strongly on the brain region studied and the dose used.

Changes in turnover rates of various catecholamines have been claimed, but methodologic difficulties prevent firm conclusions,[36] and later studies found no effects on NE turnover. The uptake of NE and DA into rat cortical preparations is markedly inhibited in vitro[57] and in vivo[29] by SNA; interestingly, SNA is 100 times more potent than ketamine in this respect. In vivo, SNA is a competitive inhibitor of striatal DA uptake and hypothalamic NE uptake in rats.[29] Tyrosine hydroxylase is inhibited and the catecholamine metabolite homovanillic acid is decreased after acute SNA administration; the latter effect is lost during chronic treatment. Adenylate cyclase and phosphodiesterase activities are decreased.[55] However, most effects appear to involve DA. SNA increases the accumulation of 3-methoxytyramine, and that indicates that the synaptic concentrations of DA are increased. SNA also has been shown to affect the release and uptake of DA from brain slices in vitro.[5] SNA decreases DA receptors in the brain. Behavioral studies have indirectly implicated the involvement of the DA system.[27] In general, effects on NE and epinephrine neurons appear smaller than those on DA neurons. It might be concluded that one of the main actions of SNA is on the DA system.[47] This might lead one to be concerned about the possibility that a parkinsonian syndrome will develop in later life; however, that seems unlikely.

The effects of SNA on the acetylcholine (ACh) system have been less well studied.[36] It has been claimed to raise rat brain ACh content slightly and transiently when given intravenously;[17] however, other studies reported no effects on rat brain ACh content concentrations when it was given intraperitoneally.[23] SNA does not release brain ACh. It has been shown to increase the activity of acetylcholinesterase in whole brain and to decrease choline acetyltransferase (CAT) activity in the cerebellum[56] while stimulating CAT in the hippocampus.[53] The effects on CAT disappear during chronic treatment. SNA has been shown to interact with muscarinic, nicotinic, and opiate receptors.[64] It inhibits the potassium-stimulated release of ACh from slices of the striatum, but not from the hippocampus. Some evidence suggests that this inhibition of ACh release is mediated via DA. At the neuromuscular junction, SNA blocks neuromuscular transmission, prolongs the action potential, and potentiates muscle twitch.

SNA does not affect the concentrations of histamine and glutamic acid, but it decreases the activity of glutamic acid decarboxylase and reduces the concentrations of gamma-aminobutyric acid (GABA) in rat brain.[25] GABA release is inhibited by acute administration, but not by chronic treatment.[25] SNA increases serum creatinine phosphokinase content in stressed rats. Recently, SNA was found to decrease methionine-enkephalin content in the medulla oblongata and midbrain in the mouse, whereas other areas remained unaffected.[48]

SNA seems to block K^+ conductance specifically.[1] It also affects ion movements and has been shown to be a potent blocker of both the K^+ channel (EC_{50}, 2.6 μM) and the Na^+ channel (EC_{50}, 9.2 μM).[1,63]

In summary, SNA administered acutely has potent pharmacologic and toxic properties and thus must have pronounced effects on biochemical events in the brain. These actions are thought to occur predominantly at the synaptic level. SNA seems to affect a number of neurotransmitters, biochemicals, and enzymes in the brain. Most evidence points to an action on the dopamine and acetylcholine systems. These changes acount for observed abnormalities in behavior and awareness. In addition, SNA blocks excitable membrane channels associated with impluse conduction, as do other compounds that induce reversible circumoral and distal paresthesias. However, neurochemical changes seem to revert to normal after the drug leaves the animal.

Unfortunately, results of SNA studies are often inconclusive or even contradictory, and the nature of the exact actions of SNA on chemical characteristics of the brain is still poorly understood. Some of the reasons for the conflicting results might be the use of acute vs. chronic administration, the use of different doses, the use of diverse animal strains and species, and the selection of specific brain areas. In addition, the results of in vitro experiments cannot be easily extrapolated to the in vivo situation, because the high concentrations necessary to produce in vitro effects are not accumulated by the brain in living organisms. However, the main objection to extrapolating these data to man might well be that all studies use healthy, undisturbed rats, whereas, as is well known, SNA produces its most serious effects in people who are emotionally labile, have had psychiatric problems, or are polydrug users.

SNA, an arylcyclohexylamine, belongs to a class of drugs known as dissociative anesthetics. Under the proper conditions of dosage and administration, it can act as a central nervous system (CNS) stimulant, a CNS depressant, a hallucinogen, an analgesic, or combinations of these.[14] Related arylcyclohexylamines share some of SNA's pharmacologic actions. The drug was originally developed as a general anesthetic agent, but it proved unsuitable for human use, because delirium often occurred as patients were emerging from anesthesia.

SNA had advantages as a surgical anesthetic, however: it can produce anesthesia with marked analgesia in the presence of normal pharyngeal and laryngeal reflexes, and it stimulates, rather than depresses, the respiratory and cardiovascular systems. It has therefore been useful in veterinary surgery, although recently its desirability as a drug of abuse has led to termination of its use by most veterinarians. Ketamine, a phencyclidine analogue with briefer duration of action, is currently in wide use as a surgical anesthetic.[79]

The pharmacology of SNA and related components has been reviewed recently.[22,51] Early in the study of SNA, it was learned that its overt actions varied enormously from species to species--thus, it produced stimulation in rats, convulsions in dogs, and tranquility in rhesus monkeys.

SNA has multiple effects on several neural systems, but evidence is increasing that at least some of its effects are mediated via interaction with a specific brain receptor. Two laboratories[65,72] have independently identified SNA binding sites in rat brain membranes. These binding sites show specificity and saturability. Other drugs with affinity for these binding sites have behavioral effects similar to those of SNA, and their binding affinity correlates well with behavioral potency. The distribution of the binding sites is similar to that of the sigma subcategory of opioid receptors, and other sigma agonists have substantial affinity for SNA binding sites. Several laboratories have recently been searching for an endogenous ligand for the binding sites/receptors; however, no positive findings have been published. Although these developments hold great promise for understanding the actions of SNA, further work is needed to clarify its complex actions.

The pharmacologic aspects of SNA that make it desirable as a drug of abuse are unclear.[41] The effects depend on dosage, but usually include sedation (light-headedness, ataxia, and slurred speech), cognitive distortions (disorganized thoughts, feelings of sensory isolation, a sense of unreality, and loss of ability to distinguish self from surroundings), and perceptual disorders (visual and auditory hallucinations and numbness). Many users become appropriately frightened or angry. Delusions of persecution may occur, and violent behavior may be directed at oneself or at another person or an object. At higher doses, SNA's effects may progress from severe agitation through stupor to coma and death. Autonomic effects are striking. SNA produces tachycardia, hypertension, hypersalivation, sweating, and fever. The acute effects persist for 4-6 h.[20]

Hallucinogenic drugs like LSD are not reinforcing in animals, in that they will not self-administer these drugs. SNA, however, is self-administered in large quantities by monkeys equipped with intravenous catheters when access is unlimited. Tolerance of the acute effects of SNA was shown to develop in monkeys and has been clinically reported in man. After SNA is self-administered by monkeys for 20-30 d, abrupt cessation produces physical and behavioral signs of withdrawal.[7,54] Thus, there is evidence of both tolerance of and physical dependence on SNA, but it is not clear that persistent daily use produces dependence in human subjects.

SNA produces an acute psychosis with some similarity to that seen in schizophrenic disorders.[35] In some users, this schizophrenialike psychosis persists for weeks after the ingestion of the drug.[2] In some cases, a patient's first psychotic episode has been precipitated by SNA and has been followed by long-term schizophrenic symptoms without additional SNA. However, results of predrug examinations of these patients were not available. SNA has been given experimentally to known schizophrenics, who were found to be very sensitive to the induction of long-lasting psychotic symptoms by the drug. It is not known whether single doses of SNA can produce long-lasting psychotic behavior in normal persons or whether this can occur only in persons with pre-existing mental disorders.[42]

Chronic users of large doses of the street drug PCP containing SNA have been reported to undergo personality changes, cognitive impairment, and slurred speech for 6-12 mo after stopping the drug. Clinical reports have included the presence of chronic anxiety, depression, and schizophrenic disorders in former long-term SNA users. Again, these reports were based on users examined after their experience with the drug, and we have no knowledge of their predrug condition.

GENETIC AND REPRODUCTIVE EFFECTS

Mutagenicity

Information on the mutagenic potential of SNA is almost completely lacking. A single abstract reported the cytogenetic effect of chronic use of the drug over 2-3 yr by one male and four females 18-26 yr old. The most frequent route of administration was inhalation of smoke from a blend of SNA and marijuana. No chromosomal abnormalities were found in this limited investigation.[28]

A cytogenetic study was performed on a group of drug-using parents of children with limb-reduction defects or of liveborn triploid infants. SNA as contained in the street drug used was implicated. An increase in ploidy was observed that decreased in time when successive peripheral lymphocyte samples were taken. The published abstract contained no specific data.[67]

Given the lack of critical studies of this chemical, no conclusion can be made as to its mutagenic activity. To determine the mutagenic potential of SNA, a battery of available in vivo and in vitro studies should be performed, including cytogenetic studies in cultured mammalian cell lines and in vivo bone marrow cells from rodents. In vitro microbial assays with and without activation should be undertaken, to determine whether this chemical causes gene mutations. In vivo and in vitro repair studies would also be informative. Additional studies, including the dominant-lethal assay and body-fluid analysis in rodents and in vivo and in vitro adduct formation, could be undertaken. These studies, with cell transformation studies in mammalian cell lines, would also indicate the probability of carcinogenic effects.

Teratogenic-Reproductive Effects

Walker and Seig[67] reported two clusters of anomalies, limb-reduction defects and liveborn triploid infants born to parents involved in the drug culture. Retrospective investigation suggested that the mothers had ingested street drugs containing SNA during the early first trimester of pregnancy.

Acute placental transfer of [^{3}H]SNA was studied in pregnant rabbits and mice; the chemical readily crossed the placental barrier. In mice, peak concentrations were found 2 h after administration, and fetal concentration was 10 times as high as that in maternal tissue. n lactating mice, the chemical was found in breast milk at a concentration 10 times that found in plasma.[50]

Jordan et al.[38] reported significant numbers of gross anomalies--including dysplasia of the extremities, micrognathia, cranial dyplasia, and cleft palate--after SNA was given intraperitoneally to rats on days 6-15 of gestation.

Summary

No definitive studies have been conducted in humans or experimental animals to determine reproductive or genetic effects of SNA. Anecdotal studies (e.g., that of Golden et al.,[30] who, in a case report of an infant whose mother used SNA, speculated that SNA was related to abnormal behavior and development of dysmorphology and spastic quadriparesis in the infant) and the limited investigations reported here indicate that this chemical may be teratogenic. If SNA is shown to be teratogenic, further studies would have to be conducted to determine whether the teratogenic effect has a genetic basis.

DELAYED AND LONG-TERM EFFECTS

SNA, in its brief use as a dissociative anesthetic in humans, produced an excessive number of cases of emergent delirium and postoperative behavioral disruption. It was eventually withdrawn from medical practice and from veterinary practice in most states. Since the mid-1960s, as PCP, a street drug of unknown purity, it has been a drug of abuse and has been used as an adulterant in other street drugs sold as cocaine, tetrahydrocannabinol (THC), and a variety of hallucinogens. Its illicit use and associated mortality rose sharply during 1975-1981. During the last 3 yr, mention of PCP abuse and related mortality has decreased, according to the DAWN system quarterly reports (Drug Abuse Warning Network, NIDA, Rockville, Md., 1982). It must be recalled that most PCP consumers never come to the attention of health-care personnel.

Information about long-term effects of SNA is derived from two sources: its administration as an anesthetic and its chronic use as a street drug. As to the latter, it must be noted that PCP rarely occurs in a pure form and commonly is abused with other substances, such as THC and cocaine.

Data on drug abusers do not permit the construction of a dose-response curve. However, blood and urine concentrations of SNA after emergency hospitalization may permit some extrapolation to the quantities consumed.[24]

Some 30 phencyclidine-like compounds are known to produce psychophysical effects resembling those of SNA. Of these, ketamine (Ketalar, Ketaject), a chlorophenyl methylaminocyclohexanone, is currently used fairly widely as a dissociative anesthetic in humans. Therefore, its use can provide clinical information about undesired effects of single doses of the phencyclidine series.

A number of possible long-term or delayed effects of SNA can be listed on the basis of experience with adverse reactions in drug abusers and in anesthesiology patients.

- Possible psychotic reactions.

- Organic brain damage as a residuum of status epilepticus or cerebral hemorrhage secondary to acute hypertension resulting from the toxic effects of SNA use.[10]

- Residual cerebral or other trauma in connection with the intoxicated state.

Prolonged Psychotic Reactions

A schizophreniform psychosis can follow administration of single or multiple, usually high, doses of SNA. Allen and Young reported on nine patients seen over a 13-mo period at an Army hospital with SNA psychosis within a week of multiple exposures.[2] Despite antipsychotic treatment, three had not recovered from the residua of their psychosis 30, 60, and 90 d later.

Erard *et al.*[25a,43] believe that the psychosis is a special form of an acute schizophrenic episode activated by the drug in some susceptible persons. Luisada estimated that 1-5% of the population may be susceptible (Luisada, P.V. personal communication). Although the Army volunteers were psychologically screened, preschizophrenic test subjects may have been included. As noted, the psychotic reactions associated with SNA typically occur immediately or soon after consumption of the drug. If serious mental consequences were not observed during the immediate followup period or during the later Army tour of duty, it seems unlikely that a delayed SNA psychosis occurred.

That a low dose was administered only once or a very few times in the Army research does not completely rule out the possibility that a vulnerable subject might have developed a delayed psychotic reation. The dose given by mouth in Edgewood experiments was up to 5 mg--a modest amount, but one that should produce some symptoms of intoxication in most nontolerant subjects.[18] Other subjects inhaled unknown amounts in aerosol form. Table 3-4 lists the clinical effects of various doses of SNA. Doses of 250 mg and more have been ingested by chronic users after the development of tolerance. In comparison, 5-10 mg intravenously can result in delirium, 20 mg in coma, and 50 mg in convulsions.

Organic Brain Damage

Case reports indicate that chronic organic brain impairment has resulted from frequent, intensive SNA use.[3,18,26] Carlin *et al.*[10] studied 12 chronic users, a like number of polydrug users who did not use SNA, and a group of non-drug-using controls. All the drug users had been abstinent for at least 3 wk. The average length of abstinence of the SNA group was 27 mo. Six in the SNA group, five in the polydrug group, and none in the control group showed mild neuropsychologic impairment. The most frequent effects were difficulties in abstracting and impairment of complex psychomotor skills.

In the amounts given to the experimental subjects at Edgewood, it is highly unlikely that major chronic organic brain syndrome developed, inasmuch as no status epilepticus or cerebral hemorrhage

TABLE 3-4

Clinical Effects of Various Oral Doses of SNA[a]

Dose, mg	Effects
5-10	Ataxia, nystagmus, mood changes, hallucinations, vomiting, analgesia, paresthesias; onset, 1-2 h; duration, 4-8 h
10-20	Stupor, eyes open, random movements, resting horizontal or vertical nystagmus, hyperreflexia, hypertension; onset, 0.5-1 h; duration, 8-24 h
50	Deep coma, chills, nystagmus, eyes open or closed, hypertension, labored breathing, seizures; duration, up to 4 d
100	Lethal; 3-10 d of respiratory depression, hypertensive crisis, cerebral bleeding, loss of deep tendon reflexes, decreased renal and liver function

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[a] Data from Hollister;[3] Vehicle unknown

occurred after drug administration. However, the development of more subtle neuropsychologic impairment, such as difficulties in cognitive functioning and the impairment of complex psychomotor skills, cannot be ruled out, because these were not evaluated.

Residual Trauma in Connection with the Intoxicated State

The behavioral toxicity that can accompany SNA intoxication can lead to head and other injuries and perhaps leave permanent residua. Such injury would occur during intoxication or delrium and would have been recorded in the Edgewood subjects' charts. No such incidents were recorded.

Food and Drug Administration Reports of Adverse Reactions

Only two reports are available on neuropsychiatric complications reported by anesthesiologists to the Food and Drug Administration in connection with SNA. One involved convulsions, and the other, psychosis. In addition to SNA, ketamine produced untoward reactions. It is clear that psychosis, hallucination, and convulsions are the most frequent immediate complications.

These FDA reports did not include the doses of SNA or the duration of the adverse reactions. Ketamine doses ranged from 80 to 575 mg intravenously or intramuscularly in the cases with postoperative adverse reactions.

Neither the FDA (for proprietary reasons) nor Parke, Davis and Co. provided any other information about SNA despite requests.

Summary

The target organs that may be involved in prolonged or delayed effects of SNA exposure are the brain and cardiovascular system. At high doses taken over a prolonged period, the effects on the brain are immediate and transitory, but occasionally a prolonged psychotic state is recorded. Neuropsycholologic effects include psychosis, a paranoid state, convulsions, and depression. Irreversible effects, such as organic mental disorders, might be encountered after frequent consumption. A prolonged or irreversible effect might be seen when a schizophrenic process is precipitated after exposure to SNA. The cardiovascular effects of acutely administered high doses include hypertension and tachycardia. These are transient and do not leave permanent residua, except after a cerebral hemorrhage.

At the dosages used at Edgewood, it is not expected or likely that any long-term or delayed effects have occurred or will occur, because immediate prolonged mental or cardiovascular effects did not take place within a week of drug administration. However, no follow-up clinical data are available to confirm this. Therefore, the development of more subtle neuropsychologic impairment, such as difficulties in cognitive functioning and impairment of complex psychomotor skills, cannot be ruled out, as these were not evaluated immediately after administration of the test drug and cannot now be evaluated in the framework of this study. Results from the questionnaire now being analyzed may shed light on some of these matters.

EFFECTS ON VOLUNTEERS

The following summary of acute effects in humans is based primarily on a review of the charts in the clinical files at Edgewood. Like many clinical records, these vary greatly in extent of detail, ranging from sketchy and incomplete notes or one-line summaries to records that could serve as models for research documents. Independent chart reviews by NRC scientists provided some cross-checks on the completeness of data. In addition, technical reports prepared at the time of the experiments provided summary data on the SNA experiments.

The SNA research going on throughout the world at about the time of the Edgewood studies is relevant to this study. The experiments at Edgewood were not unusual and did not involve extraordinary doses (see Table 3-1) or special populations of volunteers, compared with experiments in the laboratories and clinics of scientists and clinical researchers throughout the world.

When the Army began experiments at Edgewood with SNA in the late 1950s, a fair amount was already known about it, because of the clinical studies sponsored by Parke, Davis. Reports published between 1958 and 1972 described the effects of SNA given to more than 1,500 people. Most were surgical and obstetrical patients given large, generally anesthetic doses ranging from 8 to 70 mg intravenously. A substantial number of children received SNA, sometimes repeatedly, for anesthesia during burn-dressing changes or tonsillectomies. Over 200 psychiatric patients, including over 100 schizophrenics, were given doses averaging about 6 mg (3-18 mg) intravenously, orally, or intramuscularly. Over 100 normal volunteer research subjects representing a variety of student populations were given average doses of 8 mg intravenously or orally. Multiple doses were given to psychiatric patients to facilitate therapeutic sessions. Repeated doses were given chronically to psychiatric patients for periods of 4-6 wk.

Of seven subjects who underwent a water loading test, those treated with SNA at 0.1 mg/kg before a saline infusion showed evidence of inhibition of antidiuretic hormone secretion. Another group of subjects were tested on the treadmill at high temperature (100°F) before and after receiving oral doses of 5 and 10 mg of SNA. SNA at these doses did not significantly add to the exercise-induced heart-rate changes, metabolic rate, evaporative heat losses, or tolerance times and in general did not interact greatly with the temperature stress.

The only studies done at Edgewood that were not similar to those done in many civilian settings involved the administration of SNA by inhalation. SNA was dispersed in a methylene dichloride solution and delivered to the subjects as an aerosol in a small inhalation chamber. Nineteen volunteers were exposed to SNA and nine others to SNB for periods of 0.5-3 min. The concentration varied, but averaged 100 $mg \cdot min/m^3$, to produce a retained dose of approximately 100 μg/kg. At 25-62 μg/kg, subjects had feelings of unreality--dreamlike states with perceptual size changes. Both horizontal and vertical nystagmus developed. Affect and mood changed and were variable, ranging from euphoria to sullen withdrawal. Some subjects were talkative and uninhibited, others passive and withdrawn. At 100 μg/kg, the aforementioned symptoms became more intense and were accompanied by visual disturbance, blurred vision, ataxia, limb paresthesias, and memory impairment. Subjects rapidly became noncommunicative. At approximately 100-180 μg/kg, analgesia, nausea, and vomiting developed rapidly. Four subjects were given the largest dose (225 μg/kg); collapse and prostration occurred in three and incapacitation in the fourth, and convulsions did not occur. They recovered over the next few hours. In these aerosol studies, dose was measured in milligrams of a 2- to 5-μm aerosol per minute per cubic meter. It was estimated that the anesthetic dose was about 20-50 $mg \cdot min/m^3$. Exposures were 250-888 mg/m^3 for the most part over 3-4 min, with Ct's of 249-2,955 $mg \cdot min/m^3$.

In these experiments, as in most of the others at Edgewood, routine biochemical tests were used to screen for unexpected hepatic or renal toxicity. No clinically significant abnormalities were noted in the records.

In summary, the Edgewood studies of SNA demonstrated a dose-dependent effect on a number of systems. Blood pressure increased, respiratory minute volume increased, and heart rate increased with no evidence of arrhythmia. At moderate doses, reflex activity was not impaired. An outstanding effect of the drug was amnesia, which was one of the properties that first attracted investigators' attention to it. Excitation and arousal occurred in some subjects and sedation, depression, and withdrawal in others at the same dose range--a well-known variability in response to SNA. Body boundaries were altered, feelings of estrangement and loneliness occurred in some, and an alcoholic-like euphoria occurred in others. Thought was generally disorganized, with an inability to maintain set. Some subjects appeared catatonic and reported dream-like experiences at the highest doses. Performance in tasks that involved sequential and organized thinking was impaired. Perceptual discriminations were altered. Psychomotor retardation was evident, with distractibility and slowing in task performance. No subjects became overly assertive, hostile, or unmanageable. In general, the signs and symptoms were relatively short-lived, disappearing at 6-8 h, although at the highest doses in occasional subjects symptoms persisted for 24 or 48 h, or even longer. The variation in response observed from the beginning of SNA research in civilian studies was evident in the Edgewood studies.

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REVIEW OF AVAILABLE INFORMATION ON DIBENZOPYRANS: DIMETHYLHEPTYLPYRAN AND RELATED COMPOUNDS

CHEMISTRY

The structure of the active principle of the cannabis plant (Cannabis sativa), Δ-9-tetrahydrocannabinol, or THC (Figure 3-2), was elucidated in the mid-1960s.[11,22,42] However, Adams and Baker[2] and Todd and co-workers[12] in 1940 independently synthesized a Δ-6a,10a congener that differed from the natural material by the position of the cyclic double bond. A series of homologues of that congener was prepared in which the alkyl side chain on the aromatic ring was varied, and the compounds were tested for their capacity to produce ataxia in dogs.[3,5] The most active compound was dimethylheptylpyran (DMHP), the 1,2-dimethylheptyl analogue (EA 1476). Table 3-5 shows the relative potencies of several natural and synthetic dibenzopyrans.[3,5]

TABLE 3-5

Relative Potencies of Several Dibenzopyrans[4]

Compound	Potency (Capacity to Produce Ataxia in Dogs)
Synthetic C_5H_{11}	1.00 (standard)
Acetate (natural THC acetate)	14.6
Natural THC by hydrolysis of acetate	7.8
Natural THC from cannabidiol	7.3
(DMHP)	512

Because DMHP contains three asymmetric carbon atoms, it can exist as four diastereoisomeric racemates, each consisting of a (+) and a (-) isomer. These isomers, synthesized by Aaron and Ferguson[1] and isolated as the acetates, have been assigned the designations EA 2233-1 through EA 2233-8; the racemic mixture was designated EA 2233 (Figure 3-2). A mixture of isomers 2 and 4 was designated EA 2233-24. Table 3-6 lists the optical rotations of the isomers.

DMHP is a colorless or pale yellow viscous oil that readily undergoes autoxidation. It is insoluble in water, but soluble in alcohols, benzene, and nonpolar solvents. Because the acetate form (EA 2233) retains the atactic potency of the parent compound in dogs and is more stable, it and its stereoisomers were a focus of attention at Edgewood and were tested on human volunteers.

The DMHP used in these tests was procured from Shell Development Corporation or made at Edgewood. Analytic information on the test material is not available, but some Edgewood-prepared samples were reported to have a purity of 98%.

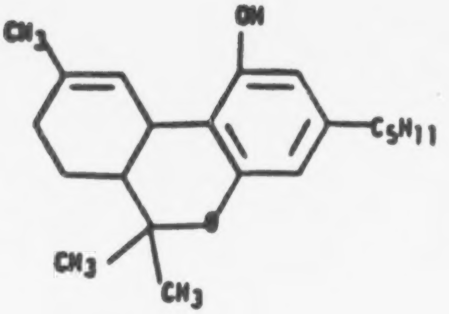
EA 2233 was produced at Edgewood, but no specific analytic information on the material used is available.

The eight optical isomers of EA 2233 were synthesized at Edgewood and analyzed by gas-liquid chromatography. Each of the eight products exhibited three peaks; a primary product in the first peak represents about 85% of the total mixture, a second peak represents about 10-22%, and a third peak represents 3-5%. The impurities were not identified, but they were not isomers.

ABSORPTION, FATE, AND ELIMINATION

Absorption

Early research on effects of DMHP and its acetate ester (DMHP acetate) was conducted in a variety of animals (mice, rats, rabbits, cats, dogs, and monkeys) with intravenous administration.[44] Rapid onset of such signs as ataxia, mydriasis, generalized weakness, nystagmus, and ptosis was seen with this route. With oral administration, fatigue, thirst, headache, postural hypotension, temporary blurring or loss of vision, and pronounced psychomotor activity were observed in humans.[44] These results indicate that both drugs are well absorbed when administered orally. Efficacies of the drugs when administered parenterally and orally have been confirmed by additional studies in cats,[8] rats and rabbits,[32] mice,[29] and man.[28,31,35,36,38,40]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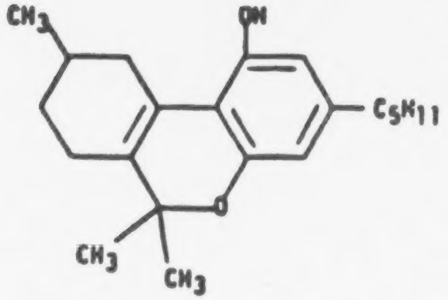
Δ-9-tetrahydrocannabinol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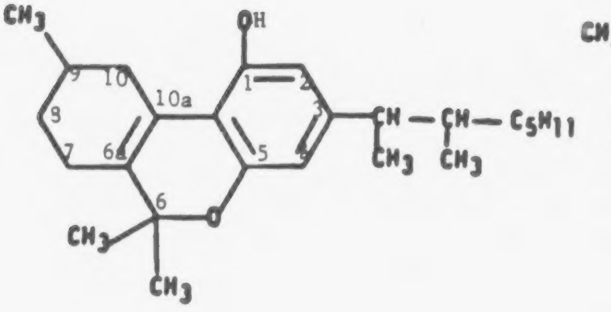
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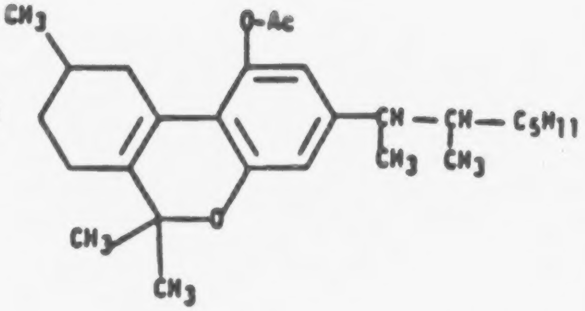
III

Δ-6a, 10a-Dimethylheptylpyran (DMHP) EA 1476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O
CH3
CH3

IV

EA 2233, Acetate form of EA 1476

FIGURE 3-2 EA 1476 and EA 2233, derivatives of Δ-9-tetrahydrocannabinol tested at Edgewood.

TABLE 3-6[1]

Optical Rotation of Isomers of DMHP Acetate[a]

Isomer	[α]D, deg (MeOH)
1	+65
2	-130
3	+133
4	-70
5	+94
6	-110
7	+105
8	-93

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[a] Isomers 1 and 4, 2 and 3, 5 and 8, and 6 and 7 are enantiomorphic pairs.

Distribution

Tissue concentrations were measured in rats and rabbits sacrificed at selected times after intravenous administration of [^{14}C]DMHP.[32] Radioactivity (from DMHP and its metabolites) in rats at 1 h after administration was highest in lung and liver. Concentrations decreased to approximately 10% of initial values in 24 h and gradually diminished during the next 48 h. Radioactivity was lower in brain than in other tissues assayed. Plasma concentrations of [^{14}C]DMHP decreased from an initial value of approximately 5,000 dpm/ml to about 50 dpm/ml at 72 h. Disappearance was in two phases; the initial one was attributed to tissue distribution (breakpoint, 6 h), and the second to elimination. Rabbit spleen, liver, and lung exhibited their highest radioactivity 3 h after injection; radioactivity in all tissues diminished with time. In this species, initial disappearance from plasma (and, presumably, distribution to other tissues) was more rapid, and the secondary phase slower. The investigators concluded that the tissue distribution of total radioactivity (from both DMHP and its metabolites) is similar to that of THC. The octanol-water partition coefficient was 7,500, compared with 6,000 for THC.

[^{3}H]DMHP was injected intravenously into adult male albino Tuck strain No. 1 mice;[29] 20 min after administration, the blood:brain concentration ratio was 3.7:1. Brain concentration of the drug, on the basis of radioactivity, was approximately 2.5 picograms/gram (pg/g) 15 min after injection and about 1.7 pg/g at 90 min; it slowly decreased to nearly 1.2 pg/g after 16 h.

In three young men who received 200 μg of a racemic mixture of DMHP containing [^{14}C]DMHP,[31] radioactivity disappeared from plasma in a multiphasic manner. The rapid initial disappearance was attributed to uptake and distribution into tissues, and the later disappearance to metabolism and excretion.

Metabolism

Cannabinoids may be metabolized to both hydroxy and dihydroxy metabolites.[9] The hydroxylated compounds are not taken up in the circulation after metabolism by the liver, but are predominantly excreted in bile. It is unlikely, but not known, whether the dihydroxylated compound can be further metabolized or activated to form more reactive compounds.

Metabolism of [^{14}C]DMHP was studied in vitro with 9000 *g* liver supernatant fractions obtained from rabbits, mice, rats, guinea pigs, or dogs and in vivo in rats and rabbits.[32] Incubation for 1 h with the supernatant fractions in the presence of an NADPH-generating system resulted in metabolism of the following percentages of the total amount of DMHP: rabbit, 68; mouse, 63; rat, 44; guinea pig, 41; and dog, 34. Thus, of the species studied, the rabbit preparation was the most active, and that of the dog the least active. The rabbit preparation gave rise to three radioactive metabolites; one of these appeared to be further metabolized to one of the other metabolites. All seemed to be more polar than the parent drug, according to their behavior in thin-layer chromatographic systems.

When [^{14}C]DMHP was administered intravenously to rats or rabbits, it appeared that DMHP was hydroxylated and then further oxidized to at least one acidic compound, which was recovered from urine. No nonmetabolized DMHP was found in the urine of either species.

A similar in vitro system used [^{3}H]Δ-9-DMHP*; mass spectra of incubation extracts were silylated and subjected to gas chromatography/mass spectrometry. Strong evidence was accumulated that the major metabolite was 11-hydroxy-DMHP. Overall recovery of the metabolite was only 4.7%; this low yield was insufficient for confirmatory analyses by other methods, such as nuclear magnetic resonance. The low recovery indicated to the investigators that DMHP and its metabolites are much more strongly bound to tissue components than are THC and its metabolites. Sixteen hours after injection of [^{3}H]DMHP into mice, their brains were extracted. Gas chromatography of the extracts indicated retention times identical with those of synthetic 11-hydroxy-DMHP, which accounted for 90% of the radioactivity; two other fractions were noted, which the investigators referred to as "non-extractable material" and "polar metabolite."

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* This DMHP is a Δ-9 form, in contrast with the DMHP used at Edgewood and by Lemberger, which was a Δ-6a,10a DMHP.

Lemberger et al.[30] used gas chromatography/mass spectrometry and reported that the hydroxymethyl metabolite represents only two minor metabolites produced by rat liver microsomes; this suggests that with DMHP the methyl group at C-11 is not active and therefore that the 11-hydroxy DMHP would not be a major metabolite. Lemberger et al. have given evidence that the hydroxylation occurs primarily on one of the methyl groups of the side chain.

Elimination

Kinetic data on the cannabinoids are limited, but it appears that they are eliminated slowly from the body. Reported THC half-lives in human plasma have ranged from 19 to 57 h. Because of their high lipid solubility, cannabinoids and their metabolites can be sequestered in fatty tissues, and traces may be detectable for over a week. The pharmacologic significance of these persistent cannabinoids and their metabolites is unknown.[26]

Very slow elimination (or at least displacement or metabolism at bioactive sites) was suspected in early studies of the effects of DMHP and DMHP acetate on human volunteers, because such signs and symptoms as sluggishness, inability to concentrate, dimness and blurring of vision, and orthostatic hypotension occurred up to 48 h after drug administration.[44] At high doses of DMHP, cats "lay in plastic-like poses for hours or days against the side of the container in which they were placed."[8]

DMHP in plasma appears to have a half-life of 20 h in both rat and rabbit;[32] the half-life of total radioactivity ([^{14}C]DMHP plus C-labeled metabolites) in the slower phase of elimination was approximately 24 h. In the rat, 70% of the total radioactivity of the intravenous dose was recovered in urine and feces during 72 h; 4% was excreted in urine and 66% was found in feces. A 7-d collection of urine and feces of rabbits given [^{14}C]DMHP resulted in recovery of 87% of the total radioactivity--24% in urine and 63% in feces. In mouse brain,[29] the half-life of [^{3}H]Δ-9-DMHP appeared to be about 20 h, whereas 11-hydroxy-DMHP and the "polar metabolite" seem to have (by extrapolation) half-lives in excess of 48 h. All three values were calculated on the basis of the slower phase of elimination.

Studies on human volunteers given DMHP intravenously indicated a half-life of the terminal phase of [^{14}C]DMHP elimination of 39 h; 58% of radioactivity was excreted in the feces and 11% in the urine during a 7-d collection period.[31] The investigators concluded that the plasma disappearance curve in humans obtained with DMHP is similar to that obtained with THC.

In summary, both DMHP and DMHP acetate are well absorbed after oral and parenteral administration, have a relatively long half-life, are extensively metabolized, and are excreted mainly via the feces.

ANIMAL TOXICOLOGY

The animal-toxicity data in this section, taken from several sources,[18,20,35,36,40,44] show that the compounds most studied in animals and humans were DMHP and DMHP acetate (more light- and air-stable than DMHP). Although eight optical isomers of the acetate were tested in humans, no specific toxicology data were found.

Route of Administration and Vehicle

The intravenous route of administration was usually used for testing these compounds in animals. Because they are insoluble in aqueous solvents, they were dissolved in small amounts of alcohol or emulsified with an oil-lecithin mixture or polyethylene glycol.

Toxicity of DMHP

DMHP was chosen as the prototype of the various cannabinol test compounds used at Edgewood and was studied more thoroughly than its congeners.

Acute toxicity studies have been performed in various animals: mice, rats, rabbits, cats, dogs, and macaques.[18,20,35,36,40,44] Lethal doses of DMHP are extremely high, in comparison with the small doses required to produce its pharmacodynamic effects. For instance, the intravenous LD_{50} in mice is 63 mg/kg, whereas the minimal effective dose in 50% of the animals (MED_{50}) is 0.075 mg/kg, for a safety factor of 840. The dose required to produce tranquilization in the unanesthetized dog is 0.05 mg/kg, and the minimal lethal dose is 10 mg/kg by the same route. The margin of safety in the dog is about 200. By comparison, the margin of safety of reserpine is 5.0.

The major signs of toxicity are ataxia, analgesia, mydriasis (less prominent in monkeys at lower doses), and profound central nervous system (CNS) depression lasting from several hours to several days, depending on dose. At higher doses, the CNS depression may be preceded by CNS stimulation and convulsions. Marked hypothermia at an intravenous dose of 1 mg/kg, hypotension, and respiratory depression are other significant effects of this compound. DMHP produces a marked decrease in mean arterial pressure in the anesthetized dog. This effect occurs slowly at small doses, but the latent period is considerably shortened at large doses. In the monkey, the minimal effective intravenous dose to produce ptosis and to decrease activity was 0.0316 mg/kg, whereas the minimal lethal dose was 10 mg/kg by the same route, for a safety factor of about 300.

Death after intravenous administration of DMHP in dogs is preceded by ventricular fibrillation, which may be secondary to hypothermia. However, there are no fatalities if the animals' rectal temperature is kept from falling.

Studies of the interaction of DMHP with other drugs were performed with dogs. If such compounds as cocaine, caffeine, d-amphetamine, and nalorphine are used to antagonize the CNS depression produced by DMHP, they also can produce a marked increase in toxicity and death. Death occurred during the depressed state that followed the stimulation induced by these agents.

Subchronic toxicity was studied in rats and dogs by administering DMHP intravenously over a period of a month.[18] DMHP at 0.1, 1.0, and 10 mg/kg-d was given intravenously to male albino rats (10 per dosage) for 20 daily injections over a 4-wk period. No effects were seen at 0.1 mg/kg. However, rats at both 1.0 and 10 mg/kg-d showed the following effects: gross signs of toxicity, decreased body weight gain, decreased liver and kidney weights, and decreased ratios of liver and kidney weights to body weight. In addition, the high-dosage group showed decreased food consumption and histologic lesions of the lungs. Histologic examination revealed of mild to severe pneumonitis, fatty metamorphosis of the liver, arrest of spermatogenesis, and cellular alterations of the ovaries among all dogs. No hematologic changes occurred at any dosage. In dogs, repeated intravenous doses at 0.1 and 1.0 mg/kg-d were given to mongrel dogs (three per dosage) for 10 daily injections during a 2-wk period. Transient signs of effect at 0.1 mg/kg-d consisted of moderate hyperpnea and a slight decrease in activity, which were noted after each injection. Similar signs of toxicity were seen at 1 mg/kg-d, and these generally persisted throughout the course of the study. Terminal body weights were significantly lower in all high-dosage dogs, and significant changes in ratios of organ weights to body weight occurred among both groups. Although hematuria had been observed in earlier studies in mice and cats, it was not observed in these long-term studies.

Studies with dogs and monkeys have shown that tolerance to the toxic effects of DMHP develops.[20,35,36,40,44]

Toxicity of DMHP Acetate (EA 2233)

The same types of toxicity data were gathered on DMHP acetate as on DMHP.[18]

In mice, the intravenous LD_{50} was greater than 25 mg/kg, and the MED_{50} (median effective dose) was 0.1 mg/kg, for a safety margin greater than 250. In the dog, slight hypertension was seen at 0.1 and 1 mg/kg. In monkeys trained on a visual discrimination test, minimal behavioral effects were seen at 0.316 mg/kg, and moderate to marked effects at 1.0 mg/kg.

Repeated intravenous administrations of DMHP acetate at 0.01, 0.1, and 1.0 mg/kg-d to male albino rats (10 per dosage) were carried out for 20 daily injections during a 4-wk period. Miosis was observed at the intermediate and high dosages, and lacrimation only at the high dosage. The high-dosage animals exhibited significantly lower food consumption, growth rates, liver and kidney weights, and ratios of liver to body weight and significantly higher ratios of adrenal to body weight. No changes attributable to the compound were seen in blood chemical tests or in gross or microscopic pathologic tests.

When dogs were treated with DMHP acetate at 0.01 or 0.1 mg/kg-d for 10 daily injections, no significant effect was seen at the low dosage. Marked defensive hostility developed at the high dosage--perhaps a sign of a cumulative effect. At this dosage, there was histologic evidence of glycogen storage in the liver.

MECHANISM OF ACTION

The mechanism of action of cannabinoids is not understood. Gill and Lawrence[13] have shown that some active cannabinoid derivatives produce disordering of artificial lipid membranes, whereas inactive compounds either do not have this action or actually increase membrane ordering, as determined by electron spin resonance. This suggests that the cannabinoids resemble anesthetics in their mode of action. Cannabinoids do, however, appear to exhibit selectivity. Thus, DMHP, which is active in animals and produces marked vasomotor changes in humans, does not induce subjective effects;[31] but nabilone, which is structurally related to DMHP, causes both subjective and vasomotor changes. Burstein and Hunter[7] have suggested that cannabinoids exhibit specificity in interacting with such enzymes as phospholipase A_2 and cholesterol esterase, which may mediate some of their actions.

NEUROPHARMACOLOGY

Little is known about the pharmacology of DMHP and DMHP acetate, but the reference compound, THC, has been extensively described both in humans and in animals. Many reviews and symposia have discussed marijuana and the cannabinoids.[6,10,19,26,39] The National Institute on Drug Abuse has reported to the Congress annually on research on marijuana. And the National Research Council[34] and the Institute of Medicine[23] issued recent reports on marijuana. Thus, we will briefly describe marijuana before commenting on the two compounds tested at Edgewood.

The pharmacology of THC in humans has been carefully explored. THC intake at 50 μg/kg by smoking produces feelings of giddiness and changes in time sense and the perception of visual and auditory stimuli. At this dose, subjects may behave in a "silly" manner. Higher doses (over 200 μg/kg orally) may produce nausea, marked visual and auditory distortions, feelings of unreality and depersonalization, and auditory and visual hallucinations. The perceptual changes may be associated with panic reactions. Pulse rate is increased in a dose-related manner; blood pressure decreases, particularly on standing (orthostatic hypotension); and conjunctival injection (reddening) is observed.[24] Although some subjects liken marijuana and THC to LSD, the effects of these drugs can be clearly differentiated by subjects in double-blind comparisons.[25] The cannabinoids may also increase blood pressure, particularly diastolic pressure.[15] Hepler and Frank[21] have shown that smoking marijuana reduces intraocular pressure, and Adams et al.[4] demonstrated that this property was shared by THC. This effect is thought to be due to vasoconstriction of the afferent blood vessels to the ciliary body, which causes a decrease in perfusion pressure.[14] THC also has an antiemetic action.[37] Marijuana and THC also produce bronchodilatation.[41]

The effects of THC and other cannabinoids on psychomotor performance are not easily summarized. There is clear impairment in most psychomotor tasks at high doses. The major conclusions that can be drawn are probably that moderate doses of THC have little effect on attention or on performance of very simple and well-practiced tasks. Furthermore, performance of complex tasks and tasks requiring complex processing of information is significantly impaired by THC and other centrally active cannabinoids.

Of the specific cannabinoids studied at Edgewood, pharmacologic data are available on DMHP. It was effective in producing sedation in animals at oral doses lower than 0.5 mg/kg. Several doses and routes of administration were studied, up to 1 mg/kg given intravenously. In rats, sedation occurred without initial stimulation; in dogs and monkeys, hyperactivity was followed by depression and then, at higher doses, by coma. Motor effects were dramatic, in that animals developed spastic ataxia and showed extremely active deep tendon reflexes. If the animals were handled while sedated by the drug, extensor seizures often occurred. In contrast with these results in intact animals, animals subjected to spinal transection showed mild depression of deep tendon reflexes.[17]

Autonomic changes were quite prominent in DMHP-treated animals--hypothermia, mydriasis, hypersalivation, bradycardia, and reduced respiratory rate. Several experimental manipulations were undertaken to determine the mechanism of these autonomic changes. The tentative conclusion was that the drug was acting centrally to reduce sympathetic tone.

DMHP was also found to have anticonvulsant properties. Studies conducted in mice showed that it was 8 times as potent as THC.[27]

DMHP was found to be 20 times as potent as THC in prolonging hexobarbital sleep time in mice. The drug produced EEG changes similar to those produced by morphine. In an experiment that might link some of DMHP's effects to opiate receptors, DMHP-induced sedation, ataxia, and analgesia were significantly reversed by the mixed-opioid antagonist nalorphine.[16]

DMHP appeared to have a wide margin of safety for acute toxic effects. One investigator calculated the therapeutic ratio for producing tranquilization in nonanesthetized dogs.[16] The ratio of LD_{50} to ED_{50} was 2,000:1, compared with only 5:1 for reserpine, a clinically approved drug. No long-term effects in animals after single doses were looked for, and no repeated-dose or chronic-toxicity studies of DMHP were reported. DMHP has more potent and more prolonged hypotensive effects than THC, but far fewer psychologic effects.

MUTAGENICITY, TERATOGENICITY, AND CARCINOGENICITY

Although the literature on cannabinoids is large, no information is available with regard to the capacity of DMHP and the eight acetate isomers to produce mutagenesis, teratogenesis, or carcinogenesis in animals or man.

DELAYED AND LONG-TERM EFFECTS

DMHP and a series of optical isomers of DMHP acetate, studied at Edgewood in humans, produce similar symptoms, but vary greatly in potency. The more potent isomers appear to produce postural hypotension and fewer psychologic effects than equivalent doses of THC. However, they all evoke redness of the eyes, dryness of the mouth, a sense of hunger, tachycardia, and drowsiness.[31,38] Postural hypotension is immediately reversed and blood pressure returned to normal by lying down.

Death due to inhalation or ingestion of marijuana has not been reported. Nor are there lasting ill effects from the acute use of marijuana,[45] except that the acute or chronic use of marijuana occasionally precipitates or exacerbates a schizophrenic state.[43] Isomer 2 is the most potent of the DMHP acetate isomers, being active at intravenous doses of 0.5-2.8 µg/kg. Postural hypotension was regularly noted, but euphoric responses were infrequent. DMHP has been the most extensively studied of these analogues.[44] At toxic doses of 50 µg/kg or more, postural hypotension, tachycardia, hypothermia, and lethargy were noted. Fatigue, thirst, and headaches were associated symptoms. Prolonged or delayed effects of a small number of acute doses were not mentioned in the literature.

The literature on THC is much more voluminous and may be used in this evaluation, because DMHP and the DMHP acetate isomers and THC are related chemically and pharmacologically. This cannabinoid also produces no known long-term or delayed effects, except when administered chronically in large doses.[33]

The doses of the dibenzopyrans used at Edgewood were similar to those used by other investigators. Lemberger *et al.*[30] used DMHP at 200 µg per 70 kg intravenously, for example. The severe postural hypotension that occurs when the drug is taken intravenously, intramuscularly, or orally is a limiting factor in giving hallucinogenic amounts of DMHP isomers.

Two long-term effects are theoretical considerations. One is that exposure to the cannabinoids may somehow have caused a chronic or delayed posttraumatic stress disorder. In the dosage and frequency used, this is unlikely. The postexperimental effect that was most undesirable was postural hypotension. This resulted in dizziness and faintness, from which all subjects recovered. Such a stress is insufficient to provoke a delayed or chronic posttraumatic stress syndrome, nor is there any evidence that any such syndrome occurred. A second consideration is that exposure to DMHP at Edgewood may have produced a tendency toward abuse of cannabinoids in later years. This is not possible to assess.

The target organs that may be involved in prolonged or delayed effects are the brain and the cardiovascular system. The mental effect consists of a transient or reversible psychosis, which may in rare instances result in activation of a schizophrenic process. The cardiovascular effects are postural hypotension and tachycardia. These are transitory and leave no permanent residua.

Given the absence of followup information on the effects of DMHP, the Committee cannot evaluate the possibility that the exposures at Edgewood produced delayed or long-term effects. However, information on THC suggests that such effects are unlikely to be associated with the exposures tested. In addition, clinical evaluations immediately after test administration did not indicate any acute effects likely to presage future complications or long-term sequelae.

A review of the epidemiologic aspects of DMHP is in Appendix C.

EFFECTS ON VOLUNTEERS

This review of acute effects on volunteers is based on clinical records at Edgewood. When the cannabinoid studies began at Edgewood in November 1958, much less was known about the pharmacology of DMHP than about the pharmacology of phencyclidine (SNA). The studies of the DMHP series in humans spanned the period from 1958 through 1968, with concentration in 1963-1966.

Although they are generally more potent, the DMHP derivatives had effects in the normal volunteers at Edgewood that were very similar to those later described over the last 15 yr by many research laboratories working with cannabis and THC. After administration of DMHP, there was more orthostatic hypotension than with THC or cannabis and possibly fewer subjective and mood effects. The time course appeared more variable, and DMHP's effects were often slower or more erratic in onset, particularly when it was given orally, than were those of THC. DMHP's effects also persisted longer.

In some of the earliest studies, beginning about November 1958, racemic mixtures of DMHP were given to approximately 35 volunteers at 0.5-4 mg per 70 kg of body weight. At 0.5 mg per 70 kg, fatigue, drowsiness, mild headache, and occasionally increased thirst developed. At 1 and 2.5 mg per 70 kg, postural hypotension was common, and faintness on standing was observed often. Blood pressure in a supine or prone position was normal or slightly increased. Weakness, ataxia, a feeling of giddiness, and general slowing of motor activity were common. At the highest doses, the subjects often showed marked psychomotor retardation, sluggishness, difficulty in concentrating, and blurred vision lasting for as long as 48 h after a single dose. Fewer comments were made about postural hypotension, probably because at this dose volunteers were unwilling or unable to get out of bed. Volunteers given over 2 mg of DMHP were judged to be incapable of performing their regular military duties. The intensity and duration of the hypotension, tachycardia, decrease in oral temperature, visual disturbance, subjective symptoms of thirst and dry mouth, and decreases in motor performance were generally dose-related, but their intensity varied among subjects. Thus, many of the signs and symptoms of DMHP intoxication were similar to those reported in recent years in many cannabis and THC studies of volunteers, except that DMHP was more potent and probably had more effects on the cardiovascular system.

The most extensive experiments at Edgewood were done in 1963-1966 with DMHP acetate. Approximately 100 volunteers were given doses of a DMHP acetate racemic mixture during this period. Oral, intramuscular, and intravenous routes of administration were used. Oral doses ranged from 3 to about 60 µg/kg. Intravenous doses ranged from 0.5 µg/kg to (in a few subjects) 5 µg/kg. Intramuscular doses were between 0.5 and 5 µg/kg. Most subjects received only one drug exposure, and a few had multiple exposures, but rarely more than two.

Cardiovascular effects were most notable. Tachycardia and orthostatic hypotension were seen in some subjects at almost all doses. ECGs occasionally showed such nonspecific changes as inverted T waves. ECGs documented the 6- to 10-s lag in heart-rate increase caused by DMHP acetate after standing. Many subjects felt lightheaded and faint on standing. As the studies progressed and the relationship between dose and orthostatic hypotension was better appreciated, this effect was less likely to occur. In general, oral doses produced changes in heart rate and blood pressure at 1 or 2 h and peak effects at 6-10 h. Major effects on the cardiovascular system disappeared in most subjects after 24 h, but persisted for several days in a few subjects in whom hypotension and increased heart rate occurred.

As is often observed with cannabis, conjunctival blood vessel injection was common. Body temperatures decreased, sometimes by 3-4°F. These changes were generally dose-dependent. Dryness of the mouth and throat, nasal stuffiness, apathy, and nausea were common, and their intensity was dose-related.

Psychomotor impairments were measured by such test batteries as the numerical facility, speed of closure, Purdue pegboard, and Stromberg manual dexterity tests. Anecdotal reports, both by subjects and by staff, of changes in behavior and mood generally paralleled the other symptoms. The spectrum of the effects and their intensity is similar to that commonly reported in the recent literature on cannabis studies in other volunteer populations. However, DMHP acetate seemed to elicit more orthostatic hypotension, and cannabis, a greater degree of mental effects.

The lack of evidence of severe mental or emotional disturbances, even in volunteers who were observed to experience intense and persistent cardiovascular effects, is noteworthy. Although DMHP acetate elicits far greater cardiovascular consequences than other cannabinoids, it appears to induce less severe mental impairment. It is possible that careful screening and a supportive test milieu tend to minimize the occurrence of adverse mental effects.

The acute effects of eight optical isomers of DMHP acetate given singly or in combination were assessed in about 125 volunteers. Several of these subjects had participated or were participating concurrently in other DMHP experiments. The isomers were given intramuscularly or intravenously. Some of the intravenous injections were given with the isomer diluted in propylene glycol and others with alcohol as the vehicle. Isomers 1, 3, 5, 6, 7, and 8 appeared to have little biologic activity (generally at about 0.5-10 μg/kg). Apart from nonspecific symptoms, such as pain at the injection site, subjects appeared unaffected subjectively and objectively. In one sense, this series of experiments provides some index of placebo responsiveness--minimal. Many subjects commented (as recorded in the charts) that they generally enjoyed the experiments, thought well of the staff support, and, in general, had few complaints other than about the food.

Isomers 2 and 4 and mixtures thereof had significant biologic activity. Intravenous doses of 1-2 mg of isomer 2 produced fairly intense tachycardia and orthostatic hypotension in the volunteers, as already described. The postural hypotension was marked, increases in heart rate were present but less intense, and feelings of impaired cognition and concentration and altered mood were present and dose-dependent. The volunteers seemed able to function reasonably well--if they were able to get out of bed and walk around. However, during the first few hours of intoxication, this was virtually impossible in many cases, because of hypotension. Dryness of the mouth, increased thirst and hunger, mild sleepiness, injected conjunctivas, and mild to severe hypotension are consistent with the effects of cannabis. Some charts contain comments about such observations as skin pallor on standing. These are understandable in the light of the circumstances.

During the DMHP studies, hepatic function and renal function were assessed. Although occasional borderline-abnormal results were noted after exposure, these were generally followed up and did not appear to be clinically significant. Some attention was given to EEG and ECG assessments to follow the intensity and duration of any drug-induced changes in cardiovascular and brain functions. In no instances of followup, did the effects appear to be particularly specific or clinically significant for acute or long-term toxicity.

In summary, DMHP and some of its acetate isomers produced various degrees of physical incapacitation due largely to the moderate to marked and prolonged orthostatic hypotension. Blood pressure was normal in the supine position. Mental effects of DMHP were much less severe than those of THC or cannabis at doses that produced similar degrees of orthostatic hypotension. Individual differences in intensity of response were considerable: some subjects showed little or no response at doses that produced intense symptoms in other subjects.

This pattern of variability has been commented on in the extensive civilian literature on cannabinoid research. Duration of effects also varied. With most doses and subjects, the majority of measurable effects disappeared in 24 h, although in a few instances they persisted for 2 or 3 d. DMHP and biologically active isomers of its acetate cause greater and longer-lasting orthostatic hypotension and fewer psychologic effects than THC; otherwise, they are very similar on the measures recorded during these experiments. The potencies of DMHP acetate and DMHP itself seemed relatively similar. The eight isomers of DMHP acetate varied greatly in potency. Those with biologic activity seemed similar to DMHP in their effects.

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CONCLUSIONS

The Committee found the evidence on the long-term health effects of the tested psychochemicals to be sparse.

The target organs that may be involved in prolonged or delayed effects of phencyclidine are the brain and cardiovascular system. Mental or cardiovascular effects were not observed, however, within one week of exposure to the drug at Edgewood.

One measure of the margin of safety of a drug can be estimated by considering the ratio of the lethal dose to the pharmacologically effective dose (the dose at which some detectable biologic effect occurs). On this basis, the margin of safety is large for acute intravenous, intragastric, intraperitoneal, and subcutaneous administration of phencyclidine in animals. It is somewhat smaller for inhalation of the aerosolized form.

On the basis of the scientific literature alone, it is not possible to predict whether any long-term effects would be associated with the exposures to phencyclidine used. However, at the small doses and low frequencies of administration used at Edgewood in a small number of test subjects, it is not likely that any detectable long-term or delayed effects have occurred.

Acute administration of the dibenzopyrans (dimethylheptylpyran and congeners) produced various degrees of physical incapacitation in Edgewood subjects, mainly because of moderate to marked and prolonged orthostatic hypotension. The duration and intensity of effects varied among doses and subjects. Despite these variations, there is a large pharmacologic margin of safety in the use of these compounds in animals. The dibenzopyrans produced more potent long-lasting orthostatic hypotension and weaker (but otherwise similar) psychologic effects than Δ-^{9}-tetrahydrocannabinol during the Edgewood experiments. There is no information on chronic effects of dibenzopyrans.

Evaluation of the toxicity literature and the Edgewood studies led the Committee to conclude that at the doses and frequencies of administration of phencyclidine and dibenzopyrans used at Edgewood, it is not likely that detectable long-term or delayed effects have occurred or will occur. Specific information to support this conclusion is, however, lacking.

4

IRRITANTS AND VESICANTS

BACKGROUND

The compounds discussed in this chapter (see Table 4-1) are mustard gas (H), chloroacetophenone (CN), dibenz[b,f][1,4]oxazepine (CR), o-chlorobenzylidene malononitrile (CS), brombenzyl cyanide (CA), diphenylaminochlorarsine (DM), chloropicrin (PS), nonanoyl morpholide (EA 1778), a cycloheptatriene (EA 4923), and 123 compounds involved in limited testing. H is a vesicant, pulmonary irritant, and systemic poison, depending on dose and route of entry into the body. The other compounds are irritants. Of the irritants, DM is a sternutator (a substance that causes sneezing), whereas the others are lacrimators. All are called gases in warfare use, although they may be administered as vapors, liquid droplets, smokes, or mixtures thereof. Mustard gas and irritant chemical agents, the latter often called harassing agents, were introduced to the battlefield in World War I to confuse, harass, and disable enemy troops. Since the 1920s, irritants have been used as riot-control agents by civil authorities. Of these, CN came into widespread use and is now commonly supplied in a formulation under the name Mace. Although CS was synthesized in 1928, it became known as a riot-control agent only in the 1960s, when it appeared safer than CN.

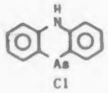
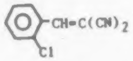
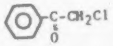
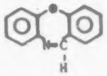
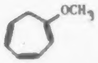
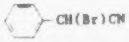
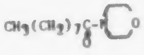
For military use, harassing agents are intended to reduce or destroy the effectiveness of enemy troops. For this purpose, rapid onset of effects is usually, but not always, desired. Rapid recovery facilitates the handling of prisoners, whereas men injured by mustard gas require intensive care and weeks for recovery. There were no plans for studying long-term effects of World War I harassing agents.

Riot-control agents are also designed to have a rapid onset of effects, produce a high degree of immediate disability, and require a short recovery time as soon as the rioters are dispersed from the area. With the increasing use of such agents as CN and CA in recent years, their possible long-term effects have aroused concern.

At the end of World War I, medical thought was turning to the possibility that soldiers who had been gassed with mustard, chlorine, phosgene, and other agents would develop tuberculosis. In the early postwar years, publications described efforts to identify cases of tuberculosis among gas casualties. The expected epidemic failed to appear, and attention subsided. More extensive studies, such as that of Beebe, were initiated.[1] Gradually, mustard gas became the

TABLE 4-1

IRRITANT AND VESICANT COMPOUNDS

Military or Common Name	Toxicity No.[a]	CAS No.	Chemical Name	Structural Formula	Type of Chemical	No. Subjects Evaluated/Tested
H, Mustard gas	E-133	505-60-2	Bis(2-chloroethyl) sulfide	$S(CH_2CH_2Cl)_2$	Vesicant	147/152
DM, adamsite	E-131	578-94-9	Diphenylaminechlorarsine		Sternutator	67/67
CS, EA 1779	E-108	2698-41-1	o-Chlorobenzylidene malononitrile		Lacrimator	1,366[b]/1,372
CN	E-129	532-27-4	Chloroacetophenone		Lacrimator	99/99
CR, EA 3547	E-124	257-07-8	Dibenz [b,f][1,4] oxazepine		Lacrimator	97/97
EA 4923, CHT	E-126	1728-32-1	1-Methoxy-1,3,5-cycloheptatriene		Lacrimator	16/16
PS, chloropicrin	E-132	76-06-2	Trichloronitromethane	CCl_3NO_2	Lacrimator	136/138
CA	E-128	5798-79-8	Brombenzyl cyanide		Lacrimator	13/13
EA 1778	E-110	5299-64-9	Nonanoyl morpholide		Pungent	32/32

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[a] Arbitrary designation by Committee staff.

[b] Including 85 given a mixture of CS and DM.

center of interest; the discovery of its mutagenic properties stimulated further research. Sufficient time has now passed for long-term effects to become clear. Mustard gas has become the subject of a large body of reports, which have disclosed a multifaceted problem.

On the other agents discussed here, there is no such volume of information. Their effects have generally been regarded as transient, and only in the Himsworth reports[2,3] on the use of CS in the Londonderry riots of 1969 is there even a proposal to study the long-term effects of the riot-control agents. Indeed, on several compounds, practically no useful information is available, except with regard to chemistry, acute toxicology, and pathology.

Because information on possible long-term effects of the other irritant chemicals used in the Edgewood tests is sparse, this chapter focuses on the effects of mustard gas and two lacrimators, CS and CN. Information on the potential long-term adverse effects of these chemicals is derived from several sources: first, observation of long-term disabilities in soldiers who were exposed to a single (in most cases) toxic concentration of irritant during World War I and in persons exposed in peacetime accidents or riot-control procedures; second, studies of morbidity in workers chronically exposed to chemical irritants during their manufacture; and third, studies in which experimental laboratory animals were exposed to selected chemicals by topical application, injection, or aerosol inhalation.

A review of the literature on experiments to assess possible chronic effects, especially mutagenic activity and carcinogenicity, of the irritant and vesicant agents reveals that these effects have not been studied systematically by current standards and techniques.

The current view is that a carefully selected battery of tests involving prokaryotic and eukaryotic organisms can be used to assess the mutagenicity or carcinogenicity of a chemical. Mutagenicity tests should include assays for gene mutations and chromosomal aberrations. No such systematic investigation has been conducted of any of the agents reviewed in this chapter, except possibly mustard gas. In the case of any agent for which a risk assessment is desired with respect to past human exposure or that continues to be used for riot control or related purposes, it seems desirable to use a battery of tests, such as those recommended in the National Research Council report on chemical environmental mutagens.[4]

Under the sponsorship of the National Cancer Institute and the National Toxicology Program, two chronic tests of CN and CS administered by inhalation are under way.[5,6] Preliminary data obtained in the subchronic studies preparatory to the definitive inhalation carcinogenicity tests are presented in the sections of this chapter dealing with CS and CN. One probable adverse effect, to judge from observation of the subchronic tests, is irritation of the upper respiratory tract and lungs. A potential consequence of inhalation of these chemicals by humans is pulmonary damage that might readily develop into pneumonia. Prediction of any other chronic effects, including carcinogenesis, awaits the generation of appropriate data, including in vitro and in vivo bioassays.

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MUSTARD GAS

CHARACTERISTICS

Mustard gas (H)--also known as yellow cross, yperite, sulfur mustard, Schwefellost, bis(2-chloroethyl) sulfide, and dichlordiethylsulfide--is a chemical-warfare agent with both vesicant and systemic effects. H is colorless and almost odorless and is an oily liquid at 14-215°C with a molecular weight of 159.08. Except in extremely cold weather, the low vapor pressure (0.072 mm Hg at 20°C) and low volatility of H are sufficient to make contaminated surfaces a source of danger to anyone nearby. H is slightly soluble in water and soluble in oils, fats, and organic solvents. It penetrates clothing readily. Moist skin of armpits, groin, and inner surfaces of thighs is especially vulnerable.[15,47,77]

Its garlicky odor, faint at first, is soon imperceptible. Exposure to H does not cause immediate discomfort; rather, the onset of effects is delayed and insidious. Troops have been known to remain in contaminated areas until their eyes, skin, and respiratory organs were affected. Exposure of skin produces erythema, then blisters that are painful and slow to heal. Such eye injuries as conjunctivitis, keratitis, and corneal ulcers cause temporary or permanent blindness. The respiratory effects of H include rhinitis, laryngitis, bronchitis, and, in severe cases, destruction of mucous membranes. The bone marrow and digestive system are affected by systemic administration of H. The multiple effects of this insidious agent make it among the most potent used on the battlefield.

TOXICOLOGY

Mutagenicity

Mutations are heritable changes in genes or chromosomes. Although mutations occur spontaneously as rare events in all organisms, their rates of occurrence can be markedly increased by exposure to mutagenic agents. Geneticists generally agree that the effects of human exposure to mutagens are deleterious and that such exposure should be minimized.[54,55] The basis for concern about human exposure to mutagens is that increases in the rates of mutation in human germ cells and somatic cells may lead to an increased incidence of genetic diseases and cancer, respectively.

After H. J. Muller showed in 1927 that x rays induce sex-linked recessive lethal mutations in the fruit fly *Drosophila melanogaster*,[51] efforts were made to determine whether chemicals can also be mutagenic. Unequivocal evidence of chemical mutagenesis was not obtained until the 1940s. Among the first demonstrations of chemical mutagenesis was a report by Auerbach and Robson[6] that mustard gas induces mutations in *Drosophila*. Over several years, Auerbach and her colleagues found that mustard gas causes genetic alterations ranging from gene mutations to chromosomal breaks and rearrangements.[5,19]

The mechanism of mutagenesis by sulfur mustard (and other mustards) involves the alkylation of DNA. As a bifunctional alkylating agent, sulfur mustard causes cross-linkage of DNA strands, as well as monofunctional alkylation products.[19] Sulfur mustard and nitrogen mustard have been used in mutation studies in a variety of organisms, but data on the relative frequencies of induction of different alkylation products in DNA by the two agents are limited. Nevertheless, sulfur mustard seems to exhibit greater S_N1 character[50] as an alkylating agent than does nitrogen mustard.[19] Because agents involved in S_N1 reactions attack oxygen sites in DNA more readily than do agents whose reactions are almost exclusively of the S_N2 type, sulfur mustard may differ substantially from nitrogen mustard in the spectrum of alkylation products formed in DNA. Such differences in mechanisms of alkylation and in alkylation products can lead to considerable differences in mutagenicity.[30,71] Therefore, although nitrogen mustard and sulfur mustard are both alkylating agents, one must be cautious in assuming they are comparably mutagenic.

A comprehensive review of the mutagenicity of sulfur mustard and nitrogen mustard has been published by Fox and Scott.[19] The mutagenicity data base on nitrogen mustard is more extensive than that on sulfur mustard. Nevertheless, results have been reported regarding the genetic activity of sulfur mustard in tests for forward mutations and reversions in bacteria;[12] differential killing of DNA-repair-deficient strains of microorganisms and their repair-proficient counterparts;[34,38] reversions in fungi;[38,68] chlorophyll mutations in vascular plants;[19] gene mutations and chromosomal aberrations in *Drosophila*;[5,19] reversions in cultured L5178Y mouse lymphoma cells;[12,19] host-mediated assays involving bacteria or mammalian cells in mice;[12] and dominant lethal mutations in mice.[62] Clastogenic effects of sulfur mustard have been studied in plant root tips and microsporocytes[19] and in cultured mammalian cells.[65]

Data from a mouse dominant-lethal test suggest that sulfur mustard reaches germinal tissue and induces dominant lethal mutations.[62] However, the data are inadequate for prediction of genetic risk to human germ cells. Uncertainties stem from the lack of data on defined genetic events induced by sulfur mustard in mammalian spermatogonia or oocytes and from the variation in mutagenic potency that has been observed for the mustards in various assay systems. Nevertheless, the possibility that sulfur mustard is a human germ cell mutagen cannot be disregarded, particularly because it is mutagenic in diverse assays, including tests for germ cell mutations in *Drosophila* and dominant lethals in mice; moreover, other direct-acting alkylating agents are known to induce mutations[63] and chromosomal alterations[21] in mammalian germ cells.

Pathogenesis of Skin Lesions

Vogt *et al*.[72] recently studied the pathogenesis of lesions caused by the application of H to the skin of guinea pigs and rabbits; their methods included light and electron microscopy, histochemistry, and the use of contrasting Evans blue dye. Cutaneous applications of H at 25-250 μg/cm^2 caused severe injury to the skin of both species and resulted in nonvesicating, necrotic, encrusted inflammatory lesions.

Cutaneous response to H had immediate and delayed phases. Within the first hour of exposure, injury to superficial capillaries and venules, vascular leakage, and infiltration by granulocytes with a high percentage of basophils were observed. The delayed phase was evident after 8 h; was manifested by nuclear pyknosis, an increase in lysosomal enzymes, and autophagic vacuoles in basal epidermal cells; and was accompanied by diffuse vascular leakage, infiltration by polymorphonuclear leukocytes, and ulceration. After the peak of inflammatory reaction at 24-72 h, the superficial, encrusted ulcers healed in about 10 d. Topical and systemic administration of glucocorticosteroids decreased the extent of edema during the immediate phase, but did not affect the rate of healing.

Carcinogenicity

Because of the correlation between mutagenicity and carcinogenicity, one would expect sulfur mustard to be carcinogenic on the basis of mutagenicity data alone. This expectation is borne out by carcinogenicity tests in experimental animals and by data from human exposures. The International Agency for Research on Cancer classifies sulfur mustard as one of relatively few chemical agents on which the data are adequate to show an association with the induction of cancer in humans.[7]

H-induced carcinogenic effects have been demonstrated in mammals. Heston[26] reported that in two experiments the intravenous injection of aqueous H into strain A mice resulted in pulmonary tumors in 93% and 68% of the surviving mice. In 1953, Heston[27] documented the appearance of a variety of tumors in strain A, C3H, and C3Hf mice after the subcutaneous injection of H in olive oil. At the injection site (middorsal area), there were sarcomas. At other sites, there were mammary and pulmonary tumors and hepatomas, and one mouse developed lymphocytic leukemia. Heston[28] found significant increases in lung tumors after inhalation exposure of mice. Heston[26-28] reported many separate experiments, some of which were performed with nitrogen mustard. Heston concluded that both mustard compounds were mutagenic and carcinogenic.

Chronic exposure of rats to H (at 1 or 100 μg/m^3, 6.5 h/d, 5 d/wk) at Edgewood produced an increase in epithelial cell tumors, but no evidence of systemic injury.[45]

The investigations discussed above show a clear progression from the discovery of a mutagenic action of H in Drosophila, through the studies of alkylation reactions of H with DNA, to the experimental production of tumors in mammals, including humans.

INDUSTRIAL AND MILITARY EXPOSURES

A few clinical observations may be instructive before discussion of the major studies of H carcinogenicity in humans.

Jackson and Adams[37] studied 33 cases of extensive basal cell carcinoma, two of which involved mustard-gas burns sustained during World War I. One of those developed 35 yr after the burn, but 2 yr after irradiation with cobalt-60. In the other, basal cell carcinoma developed at the site of three separate burns, 3 yr after exposure. Some of the mustard burns did not lead to basal cell cancer.

Illig et al.[35] treated nineteen patients suffering from psoriasis vulgaris once or twice with 50 g of 0.005% H in petrolatum. They concluded that whole-body inunction with H presents a low carcinogenic risk. That is likely to be erroneous, in view of the low dose and brief treatment used.

When examining reports of exposures to chemical agents, one should note the different conditions involved. After July 1917, during World War I, H was used often by both Germany and the Allies. Some areas of French battlefields became so badly contaminated that they were abandoned by both sides.[13] Thorpe[69] estimated atmospheric concentrations of H during gas shelling as averaging 3 ppm, with a maximum of 5 ppm (about 19 and 32 mg/m^3, respectively). Prentiss estimated that exposure at 23 ppm (150 mg/m^3) for 10 min, giving a Ct (product of concentration and duration of exposure) of about 1,500 $mg \cdot min/m^3$, would be lethal for an unprotected man.[60]

In military and riot-control situations, exposure to agents is acute, but relatively brief. The clinical cases cited[17,40] involved "long-term" exposure, meaning a few months to a few years.

Nakamura,[53] in a 1956 paper, reported working conditions in a Japanese mustard-gas factory operated secretly in Hiroshima from 1930 to 1945. Workers alternately worked 1 h in gas production and 2 h in a gas-free environment over a 10-h workday. They wore gasmasks and complete protective clothing, including rubber boots, and were often rotated. Nevertheless, many workers showed a darkening of skin; some developed ulcers, diarrhea, and jaundice and later coughed blood and developed tuberculosis. The concentration of mustard gas[78] may have reached 50-70 mg/m^3, as determined by bioassay. The bioassay involved exposure of unprotected birds in the work areas that resulted in death in 12-15 h. Exposed rabbits refused food, coughed, and died within a 3-d period. Some adverse health effects experienced by workers may have been due to the inadequacy of protective equipment.

Yamada et al.[79] found 97 deaths during the period 1946-1957 among workers exposed to H in a Japanese factory before and after the war. Of the 97 deaths, 20 were from malignant tumors, 13 of them in the respiratory system. In 1963, Yamada[78] further reported 172 deaths, extending the survey to 1933-1962. Yamada gave no figure on the total number of men, but Wada et al.[73] stated that there were 495. Forty-eight deaths (28%) were caused by cancer, including 28 (16% of the 172) involving the respiratory tract. Among 5,030 deaths in the nonexposed general population, 406 (8%) were from cancer, including 25 (0.5% of the 5,030) involving the respiratory tract (Table 4-2).

Wada et al. extended Yamada's observations on the same men,[73] finding 33 deaths from respiratory tract cancers for 1952-1967, compared with an expected 0.9, a relative risk of nearly 37. For 960 employees not exposed to H, Wada et al. found only three deaths from respiratory tract cancer, compared with 1.8 expected. These data point to a connection between long, low-dose exposure to H and later cancer, especially in the respiratory tract.

Weiss and Weiss[75] found a statistically significant increase in malignant tumors, especially bronchial and bladder carcinomas and leukemia, among 245 German workmen employed in the manufacture of H and nitrogen mustard (HN) in the period 1935-1945. The 245 men, studied over 20 yr, all had verified case histories. By 1974, 114 of these men had died, 40 of malignant tumors and 38 of chronic respiratory ailments. From 1951 to 1972, there were 32 deaths from various cancers among the exposed workmen, more than expected in a comparable nonexposed population; only bronchial carcinoma showed a statistically significant difference: 11 observed vs. 5 expected, a relative risk of more than 2.

Hellmann[25] studied German munitions workers and reported 20 deaths from cancer among 157 former workmen. It is not clear whether these were included in the 245 of Weiss and Weiss.

Morgenstern et al.[48] reported on over 200 workmen in an American chemical plant making H during World War II, focusing on 10 case histories that illustrated the immediate and delayed effects of daily exposure to small quantities of H vapor. Exposure for 3 wk to 6 mo 'ed these men to the dispensary for treatment of respiratory distress. Typically, a man developed some or all of the following symptoms: red eyes, photophobia, lacrimation, impaired vision, blepharospasm, loss of taste and smell, nosebleed, sore throat, chest pain, wheezing, and dyspnea. After several such occurrences, a worker was removed from further contact with H.

TABLE 4-2

Characteristics of Male Mustard-Gas Workers Who Died from Cancer (1955-1967)[a]

Case	Exposure Duration, yr	mo	Interval from Employment to Death, yr	mo	Age at Death, yr	Site of Neoplasm	Histologic Type
3	7	2	22	4	62	Pharynx	Undifferentiated
4	5	3	22	0	40	Pharynx	Squamous cell
5	8	0	24	5	57	Paranasal sinus	Squamous cell
6	4	11	23	2	44	Paranasal sinus	Squamous cell
7	7	5	28	1	65	Paranasal sinus	Squamous cell
10	8	0	16	10	62	Larynx	Squamous cell
11	10	0	19	2	52	Larynx	Squamous cell
12	12	10	22	11	58	Larynx	Squamous cell
13	7	11	20	10	58	Larynx	Squamous cell
15	8	0	25	5	48	Larynx	Squamous cell
16	7	0	23	4	59	Trachea	Squamous cell
18	1	4	10	11	30	Bronchus	Squamous cell
21	6	1	23	1	62	Bronchus	Squamous cell
22	8	1	17	4	62	Lung	Squamous cell
23	2	0	33	0	54	Bronchus	Undifferentiated
24	17	0	27	0	54	Bronchus	Undifferentiated
26	16	4	27	10	61	Bronchus	Undifferentiated
27	4	10	17	9	61	Bronchus	Squamous cell
29	2	2	19	3	58	Bronchus	Undifferentiated
30	8	5	22	5	63	Bronchus	Undifferentiated
32	5	8	20	8	50	Bronchus	Undifferentiated
38	7	11	27	7	74	Lung	Squamous cell
40	2	0	27	6	55	Bronchus	Squamous cell
43	0	3	26	1	47	Lung	Undifferentiated
44	7	3	29	0	63	Bronchus	Squamous cell

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[a] Data from Wada *et al*.[73]

Büscher,[11] Morgenstern et al.,[48] and others emphasized the lingering bronchitis, bronchial asthma, hoarseness, aphonia, and hypersensitivity to smoke, dust, and fumes that develop especially in men working in industrial situations that expose them to mustard at constant low concentrations. Even after discontinuing such work, they may be subject to continuing respiratory and systemic disabilities with a general deterioration of health that leaves them partial or complete invalids. These men recovered partially after leaving the mustard plant, but were subject to bronchitis, were susceptible to respiratory infections, and were likely to develop bronchiectasis. Many men were partially or completely disabled by 1945, when these observations ended. The implication of the observed disabilities is that complete recovery would probably not occur. It should be emphasized that the sequelae outlined here resulted from the chronic, long-term exposure to H in the working environment. Most important, these long-term sequelae (except the malignancies) generally constituted extensions or continuations of acute problems experienced during exposure to H; they did not suddenly appear years after exposure.

MEDICAL EFFECTS

Immediate Effects

An unprotected person exposed to H vapor will suffer simultaneously from skin burns, eye injury, and irritation of the respiratory tract.[20] The acute effects of H depend on the concentration of the gas, the duration of exposure, the ambient temperature, the extent of protection, and the susceptibility of the person.[23] Clothing will be contaminated and become a secondary source of poisoning even after a gas cloud has blown away.

Onset of action may occur within several hours of exposure or after a latent period of up to 24 h. The immediate effects of H within 0.5-3 h of exposure include sneezing, acute conjunctivitis, lacrimation, rhinorrhea or nasal bleeding, soreness and burning of the the throat, hoarseness and dry, hacking cough, and erythema of exposed skin. Within 4-16 h of exposure, the effects of H include eye pain with acute conjunctivitis, corneal edema, lacrimation, photophobia, blepharospasm, and edematous erythema of the eyelids; nausea, vomiting, diarrhea, and epigastric pain; and erythema and vesication of the skin with coalescence of vesicles to form bullae.[2,47,77] Between 24 and 48 h, effects on the eyes and skin progress and are manifested by eyelid pain and edema with blepharospasm, lacrimation, photophobia, and blindness; erythema, vesication, and edema of the skin; persistent cough and hoarseness or aphonia due to membranous laryngotracheobronchitis; increased temperature, pulse, and respiratory rate, as well as granulocytosis resulting from secondary infection or bronchopneumonia; persistent nausea and vomiting; and marked apathy, depression, and despair.[11,15,23,24,47,77] If death occurs, it is usually after 48 h and as the result of the systemic effects of H with evidence of shock or from secondary infection, depletion of hemopoietic cells in the bone marrow, and leukopenia.[2,77]

The acute pathologic effects of H on the eye include edematous clouding of the cornea and necrosis of corneal stroma. About 5 h later, infiltration by segmented leukocytes is noted at the sclerocorneal junction and in the corneal stroma. Healing usually occurs in several weeks, but the injury may result in persistent or recurrent corneal ulceration and blindness.[33]

Burns of the skin by H may range from erythematous subvesicating injuries to large annular blisters. Experimental studies[61] of humans exposed to H have shown the following pathologic effects between 12-18 h: epidermal nuclear pyknosis, lysis of cytoplasm, liquefactive necrosis in the malpighian layer above the dermal papillae, and hyperemia, edema, and perivascular mononuclear infiltration of the dermal papillae. After 12 h, the foci of liquefactive necrosis may coalesce and enlarge to form vesicles, and there the dermis is infiltrated by mononuclear cells and polymorphonuclear leukocytes. By 72 h, there is ingrowth of epithelium at the margin of vesicles with coagulation necrosis of the corium. Healing is complete in 4-5 wk.

Postmortem examinations of persons who died as a result of exposure to H have shown depletion of lymphoid cells in the spleen, thymus, and other lymphatic organs; depletion of hematopoietic cells of the bone marrow; necrosis and desquamation of epithelium in the small intestine; acute ulceration of the duodenum; membranous laryngotracheobronchitis; and pulmonary edema, congestion, and patchy emphysema that may be complicated by bronchopneumonia or other evidence of pulmonary infection.[2,47]

The acute effects of H were observed in three children, as well as in the rescuer and attending medical and nursing personnel, after they were injured by the accidental explosion of a 40-yr-old mustard-gas shell.[29] Two children died within 4 h after experiencing congestion and swelling of the eyes, continuous vomiting, edema of the skin, pulmonary edema, pain, and shock. The older child survived, but full recovery took 5 wk. Vomiting, patchy erythema of the skin, and edema of the face were noted for 3.5 h after injury. At 5.5 h, there were vesicles on the face, puffy eyelids, irritated conjunctivae, and evidence of photophobia. Coma and agitation occurred between 6 h and 5 d. The rescuer and attending personnel had delayed onset of symptoms, including drowsiness, coughing, nausea, vomiting, hoarseness, ocular pain, photophobia, lacrimation, blepharospasm, conjunctivitis, headache, dyspnea, and burns of the skin.

A German text on noxious gases[18] updates approximate exposure conditions that may result in injury from mustard gas (Table 4-3).

Injury to Skin

Except with high concentrations of H, the initial vesicating reaction may take hours, so that more of the agent is absorbed before its presence is recognized. Cullumbine,[15] describing the action of an experimental droplet on human skin, said, "The first macroscopic sign of the action of mustard gas appears under temperate climatic conditions about two hours later" (after application). Penetration of the skin is rapid, but only about 12% remains in the skin, the rest moving into the circulatory system.[15]

In 1919, vast quantities of German chemical munitions were stored in the Luneburg Heath, awaiting destruction. On October 24, an explosion destroyed buildings, tank cars of chemicals, stacked artillery shells, and other material. When workmen began the task of cleaning up, chemical burns were inevitable. Hermann Büscher,[11] a young physician just out of the German Army, practiced only a few kilometers away in Münster. At first a few, then a flood of injured workmen were sent to Büscher. Knowing almost nothing about the chemicals and their effects, he worked empirically, gradually developing systematic therapies for the results of exposure to mustard, Lewisite, and other agents.

Eventually, Büscher experimented on human volunteers. Like Cullumbine, he found that, when a drop of H is placed on skin, the initial reaction appears in about 2 h. Vesication begins in about 24 h, but healing does not begin until after about 4 wk, and later for severe burns. Büscher wrote: "There are also irreparable, permanent injuries. . . . Cicatricial contractures are very frequent sequelae. . . . Following severe wounds this scarring is very extensive so that there can be no question of complete recovery." In all cases of extensive burns from mustard gas, careful and extended treatment is required to prevent infection and other complications.

Eye Injuries

Of the long-term complications of wartime exposure to mustard gas, perhaps the best documented and one of the most serious is recurring corneal ulcers, with eventual opacification and blindness.[32] No exact figures are available for predicting the eventual development of such long-term corneal lesions, but it has been reported that a Ct of 100 mg min/m^3 will cause acute blindness for 24-48 h.[20] Permanent blindness typically occurred about 14 yr

TABLE 4-3

Effects of Various Concentrations of Mustard Gas on Humans[a]

Concentration			
mg/m^3	ppm (approx.)	Duration of Exposure	Effects
0.5	0.08	10-25 min	Eye and skin damage
1.0	0.15	1-2 h	No serious damage
1.0	0.15	8-10 h	Incapable of combat
1.2	0.18	45 min	Damage to eyes after 12 h; damage to skin after 2 d
2.5-5.0	0.4-0.8	30-60 min	Slight irritation of sensitive skin
6.5	1.0	60 min	Occasional serious lung injury
70	11	30 min	Apparently lethal
100-200	15-31	Few seconds	Itching of sensitive skin for many weeks

---

[a] Data from Flury and Zernick.[18]

after acute necrotizing injury to the corneal epithelium and basement membrane, with but little loss of visual acuity in the intervening years.[32]

The human eye is sensitive to H vapor, and liquid drops will produce severe burns leading to blindness. Inflammation, conjunctivitis, iritis, and keratitis are terms used to describe eye injuries. Temporary or permanent blindness results from light to moderate exposures to the vapor. Efforts to measure the effects of H vapor on animal eyes have yielded values cited by Hughes.[32] The human eye has been estimated to be 4 times as sensitive as the rabbit eye:[20] a Ct of 100 $mg \cdot min/m^3$ causes impairment of vision for 24-48 h, and it is estimated that a Ct of 200 $mg \cdot min/m^3$ would produce blindness for a week or more (Table 4-4).

Buscher encountered relatively few cases of liquid mustard in workmen's eyes, because gasmasks were usually worn. Of men exposed to H vapor, he wrote, "Even in cases of severe keratitis in which there are various opacities of the corneal epithelium, the disease usually terminated fairly favorably, without leaving too much permanent damage." This was written in 1931 before the delayed and relapsing keratitis recorded by later writers occurred.

Mann[43] examined the records of 84 men described as suffering from "delayed mustard gas keratitis." This group had been treated at the Contact Lenses Clinic at Moorfields, England. The eye injuries were described as "typical mustard gas scars with corneal degeneration." Mann found a low incidence of onset of trouble in the early postwar years, with a sharp rise in 1931 and peaks in 1934 and 1937 (Figure 4-1). Most, 19-23 yr old when gassed, were about 33-37 when the eye trouble peaked. The onset of symptoms was commonly provoked by minor eye injuries and followed by ulcers that tended to recur spontaneously and cause steady diminution in visual acuity. These men were all fitted with contact lenses. About half were able to wear them with improved vision; the others varied from partial success to total failure. Even those helped most, however, suffered slow deterioration of visual acuity.

Scholz and Woods[66] reviewed 136 cases of mustard injuries of the eye, including the 84 described by Mann. They found no essential difference between "chronic" and "recurring" cases. Although a number of injured men retained "fair visual acuity" after the initial burn, vision degenerated later; the average loss of vision (excluding five minor injuries) was about 88%. The only effective treatment for these patients was contact lenses.

TABLE 4-4

Dosages of H Vapor for Production of Injuries in Man

Protection	H Dosage, mg·min/m^3 Hot and Humid Weather, Temp. above 80°F, Sweating Skin	Warm Weather, Temp. 60-80°F, Skin not Wet with Sweat	Cool Weather, Dry, Temp. 40-60°F, Skin Cool and Dry	Effect	Disability	Time to Onset	Duration of Effect
None (no mask or protective clothing)	50	50	50	No significant injury, maximal safe dosage	--	--	--
				Eye damage of threshold military significance	Partial	6-24 h	1-3 d
	100	100	100	Temporary blindness	Total	3-12 h	2-7 d
Mask (no protective clothing)	100	150	400	No significant injury, maximal safe dosage	--	--	--
	200	300	1,000	Skin burns of threshold military significance	Partial	2-12 d	1-2 wk
	500	1,000	2,000-4,000	Severe genital burns	Partial	2-7 d	1-4 wk
	750	2,000-4,000	4,000-10,000	Severe generalized burns	Total (Partial)	24 h (4-12 h)	1-2 wk (3-6 wk)

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* Data from Gates and Moore.[20]

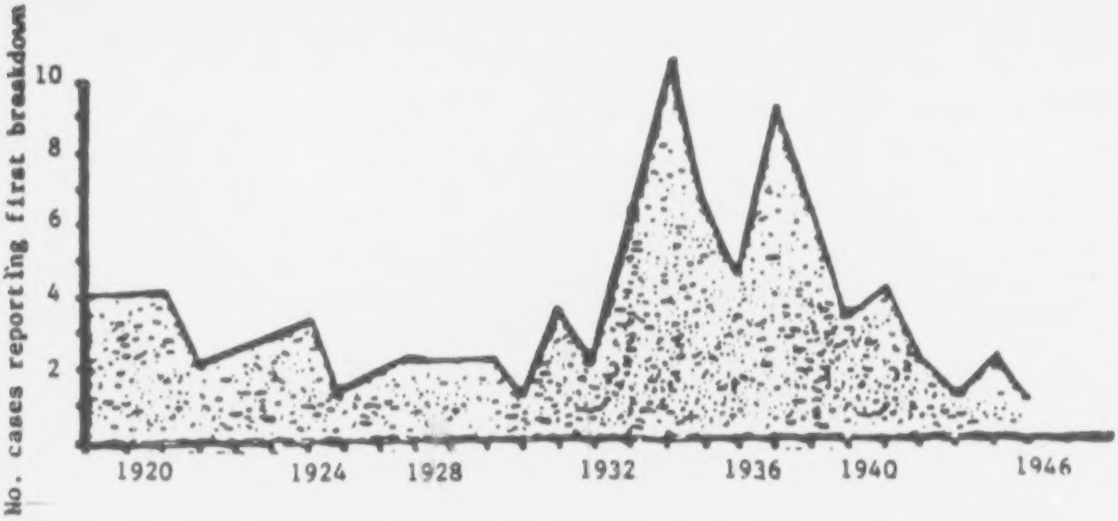

FIGURE 4-1 Distribution of onset of delayed keratitis in 82 of 84 cases. Two of the 84 did not remember the date of the first onset of trouble after apparent cure. Four stated that they had had continuous trouble from the time of gassing, and four had had a slight breakdown within a year. Redrawn from Mann.[28]

Hughes[32] reviewed mainly British and European reports and cited official British data estimating that 75-90% of mustard gas casualties had some degree of ocular injury. A rough estimate, based on information reported by Case and Lea,[13] indicates a little over 100,000 cases of eye injury. Hughes stated that about 10% of these injuries resulted in corneal erosion, which he considered predictive of visual degeneration. Corneal transplants or contact lenses could be expected to help many patients.

Hughes[33] cited these doses of H found by various experimenters to produce eye lesions:

- Destructive corneal lesions (rabbit?)--0.0004 ml.
- Ocular lesion (rabbit)--70 mg/m^3 (as vapor) for 30 min.
- Conjunctivitis (dog)--1 mg/m^3 (as vapor) for 2 h.
- Visible reaction (man)--0.5 mg/m^3 (as vapor) for 1 h.

Atkinson[4] provided details on two of his patients who had suffered mustard poisoning from the vapor; both eyes and lungs were affected. He found no decrease in visual acuity (both men being practically perfect in acuity) between prewar and 20-yr-postwar examinations. Both had some corneal haziness, which proved the precursor of progressive deterioration of vision.

Injury to the Respiratory System

Studies of fatal and near-fatal human exposures to mustard gas, with animal studies, provide compelling evidence in the aggregate that exposure to H can cause injury to the respiratory system, particularly to the bronchial tree, and that lethal doses can be associated with a superimposed bronchopneumonia with abscess formation (reviewed by Gilchrist and Matz[23]). Moreover, as many as 19,000 British soldiers, or approximately 12% of those exposed to mustard gas in combat, were pensioned during 1920 for disability believed to be caused by gas exposure during combat.[22] The predominant type of pulmonary injury reported is acute and chronic bronchitis. A factual basis for such injury is provided by repeated demonstrations in both man and experimental animals that high exposures to mustard gas cause necrosis of airway epithelium, often with pseudomembrane formation and secondary bacterial infection.[23]

Berghoff[9] noted that, whereas a single exposure to mustard gas primarily affects the respiratory tree acutely, long-term (5 mo) effects of such exposure most notably involved the skin and eyes, although as many as 30% of gas victims also appeared to manifest chronic bronchitis. Similarly, Gilchrist and Matz[23] reported that gas victims could develop chronic bronchitis, emphysema, and asthma--again, as long-term (10 yr) consequences of mustard exposure.

Less clear, however, is the exact incidence of pulmonary sequelae after exposure to mustard gas, because it was often difficult to document whether a person had been exposed to mustard alone, to mustard in combination with another agent, or to an entirely different agent.[22,42] In yet other cases, influenza or other respiratory diseases were misdiagnosed as mustard-gas injury.[8,22] It was particularly difficult to determine with any accuracy the dosage or extent of exposure under wartime conditions, particularly where people received multiple exposures. Finally, early long-term studies of chronic pulmonary effects often failed to control or correct adequately for other causes of chronic pulmonary disease (particularly bronchitis), such as cigarette-smoking and long-term exposure to polluted urban environments. In summary, although there is compelling reason to believe that high-dose exposure to mustard gas can result in acute and chronic injury to the respiratory airways (bronchitis), the data do not permit one to calculate the dose-response relationships or the incidence of pulmonary sequelae with any degree of certainty.

Injury to the Nervous System and Other Organ Systems

Injury to the nervous system, although often reported, is somewhat more subjective, and the reports do not appear to have been based on objective data gathered in a scientific fashion. The general malaise, apathy, depression (reviewed by Lohs[41]), loss of libido,[74] and other psychopathologic effects are difficult to interpret in the absence of carefully controlled studies. Other reported effects include brain abscesses, periodontal disease, osteoporosis, and liver damage, but, again, substantive epidemiologic data are lacking.

Systemic Effects

Lohs[41] reviewed the literature and came to highly disputed conclusions. The panel felt that information was misquoted, distorted, scientifically inaccurate, or politically flavored, because authors hold strong personal views opposing chemical warfare.

Cullumbine[15] postulated the production of a "capillary permeability factor" caused by the reaction of H with tissues. Experiments that he and co-workers performed supported the appearance of such a factor. Figure 4-2 shows what he suggested occurs after H contaminates the skin.

In severe poisoning, shock, vomiting, and diarrhea develop. Cullumbine's estimate of 12% retention of mustard in the skin means that nearly all the agent attacks the body generally. Citing contradictory results obtained by others, he came to no conclusions as to precisely how the mustard is carried in the circulation, opining that both formed elements and plasma are carriers and that the circulating mustard is taken up rapidly by the body tissues. Leukopenia occurred in World War I gas casualties, facilitating secondary infections and delaying recovery.

In discussing the effect of mustard on the intestinal tract, Cullumbine pointed out that severely poisoned men suffered loss of water and electrolytes because of vomiting, diarrhea, and vesication. Oligemia and irreversible circulatory failure follow, much as in thermal burns. He showed the importance of the loss of water and electrolytes by experiments in which the mortality rate of poisoned rats and rabbits was greatly reduced by the administration of physiologic saline solution either by mouth or by injection.

Long-Term Morbidity

The long-term morbidity associated with exposure of soldiers to H at toxic concentrations is well established. Although it was not possible, in most cases, to determine the Ct for each individual exposure, it has been estimated that battlefield concentrations ranged from 19 to 32 mg/m^3.[69] This estimate seems reasonable, inasmuch as approximately 1% of exposures proved fatal[23] and short

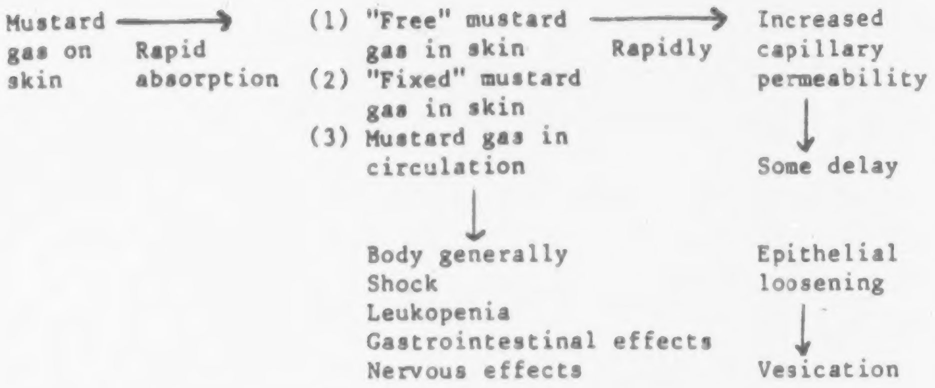
Mustard gas on skin
Rapid absorption
(1) "Free" mustard gas in skin
(2) "Fixed" mustard gas in skin
(3) Mustard gas in circulation
Rapidly
Increased capillary permeability
Some delay
Epithelial loosening
Vesication
Body generally
Shock
Leukopenia
Gastrointestinal effects
Nervous effects

FIGURE 4-2 Physiologic processes that occur after mustard-gas contamination of skin.

exposures at 150 mg/m^3 are generally lethal for man.[60] Long-term sequelae can be further evaluated from the fact that approximately 20,000 of 150,000 British gas victims were pensioned after the war; these figures include all forms of gas used, and the proportions for mustard gas are likely to be larger.

WORLD WAR I CASUALTIES: SEQUELAE

Tuberculosis

During the latter half of World War I and the early postwar years, there was serious concern that men poisoned by inhalation of chlorine, phosgene, and mustard (the three most common and deadly agents) would develop tuberculosis and cancer. Limited studies were begun, but it proved difficult to produce evidence to support the idea. Achard,[1] Wilson and Mackintosh,[76] Sergent and Haas,[67] Dennis,[16] Cowen,[14] Morris,[49] Meade,[46] Atkins and Klotz,[3] Berghoff,[9] Sandall,[64] Hueper,[31] Gilchrist,[22] Gilchrist and Matz,[23] and the U.S. War Department[70] all reported on this. No increased incidence of tuberculosis was found.

About 10 yr after men had been gassed, Gilchrist and Matz[23] found residual disabilities, such as chronic bronchitis (usually accompanied by emphysema), bronchial asthma, chronic conjunctivitis, blepharitis, keratitis, and corneal opacities.

Beebe,[8] in a later study mainly on cancer, included some data on tuberculosis. These data (Table 4-5), although suggestive of a residual effect from mustard-gas exposures, were not statistically significant.

Cancer

The work of Boyland and Horning,[10] Heston,[26-28] and others stimulated interest in the mutagenic and carcinogenic potential of mustard gas. This was followed by the work of Case and Lea,[13] who examined the possible carcinogenic effects of H on exposed British soldiers. They cited total British gas casualties as 160,970, 80% of whom were estimated to have been H casualties. As of January 1, 1930, 1,267 men in England and Wales were receiving pensions for the effects of H in 1917-1918. Almost all were suffering from bronchitis. This group was studied. A group of 1,421 men who were pensioned because of bronchitis but had not served overseas after the first use of mustard gas were controls. A second control group of 1,114 was selected at random from men not exposed to mustard gas and pensioned because of single-leg amputation. The results of this study, summarized in Table 4-6, showed unusually high mortality in both the mustard and bronchitis series. In the mustard series, the high mortality involved excessive deaths from bronchitis and, to a lesser extent, excessive deaths from cancer of the lung and pleura. In both series, deaths from cancer of the lung and pleura occurred at twice the rate for the general population. The amputation series did not deviate from the rate of the general population. Case and Lea[13] concluded that chronic bronchitis from H led to cancer of the lung and pleura, but that H was not a direct carcinogen.

Beebe,[8] like Case and Lea, set up three groups: mustard gas casualties, pneumonia cases, and those with leg wounds; the latter two series excluded any who might possibly have had contact with H. Sample size was set at 2,500 each, but the restrictions left Beebe with groups of 2,718 H casualties, 1,855 cases of pneumonia, and 2,578 men with leg wounds. All in the H group had evidence of H injury to skin, eyes, and respiratory tract. All were born between 1888 and 1893 and were alive on January 1, 1919. Records in the Veterans' Administration files enabled Beebe to follow the men up to January 1956. In a followup study of lung-cancer mortality, Norman[56] extended Beebe's work to 1965. Because Norman studied the same men, his data are presented here in combination with Beebe's.

Table 4-7 shows observed deaths in the three categories compared with age-specific expected numbers based on mortality data on U.S. white males born in 1891. In general, the observed:expected ratios are lower than 1, apparently because the preinduction medical examinations excluded men not in good health. Deaths did increase sharply in the mustard-gas roster for 1930-1939.

During the last 7 yr of the Norman study (except 1956-1958), all three rosters showed mortality lower than the standard values for all U.S. white males. Mortality from cancer of the respiratory system

TABLE 4-5

Numbers of Deaths and Deaths per 1,000 Men per Year for Respiratory Tuberculosis, by Roster and by Period of Followup[a]

	Roster					
	Mustard Gas		Pneumonia		Wounded Controls	
Followup Period	No. Deaths	Rate	No. Deaths	Rate	No. Deaths	Rate
1919-1928	17	0.64	23	1.28	15	0.59
1929-1938	41	1.63	12	0.70	13	0.54
1939-1948	27	1.20	15	0.96	19	0.86
1949-1955	14	1.03	4	0.43	7	0.53
Total	99	1.13	54	0.89	54	0.63

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[a] Data From Beebe.[8]

TABLE 4-6

Mortality from Each Class of Disease Found and Expected in Three Series Studied[a]

		Disease Class							
		All Causes		All Neoplasms		Cancer of Lung and Pleura		Neoplasms other than Cancer Cancer of Lung and Pleura	
Series	Deaths	No. Deaths	Mortality Ratio	No. Deaths	Mortality Ratio	No. Deaths	Mortality Ratio	No. Deaths	Mortality Ratio
Mustard gas	Found	547	1.53	79	1.30	29	2.07	50	1.07
	Expected	357.3	(VHS)	60.8	(S)	14.0	(HS)	46.8	(NS)
Bronchitis	Found	932	1.38	104	1.09	29	2.01	75	0.93
	Expected	673.8	(VHS)	95.0	(NS)	14.4	(HS)	8.06	(NS)
Amputation	Found	383	1.05	72	1.00	13	0.84	59	1.04
	Expected	365.7	(NS)	72.2	(NS)	15.5	(NS)	56.7	(NS)

---

[a] Data from Case and Lea.[13] VHS = very highly significant ($p < 0.0001$). HS = highly significant ($0.01 > p > 0.0001$). S = significant ($0.05 > p > 0.01$). NS = not significant ($p > 0.05$). Mortality ratios based on theoretical numbers of years lived.

TABLE 4-7

Observed and Expected Deaths, by Roster and Time[a]

Series and Period	No. Deaths Observed	No. Deaths Expected	Mortality Ratio
Mustard-gas			
1919-1929	122	163	0.75
1930-1939	248	190	1.31
1940-1949	314	323	0.97
1950-1959	519	534	0.97
1960-1965	413	426	0.97
Total	1,616	1,636	0.99
Pneumonia			
1919-1929	97	112	0.87
1930-1939	121	130	0.93
1940-1949	224	221	1.01
1950-1959	346	372	0.93
1960-1965	269	300	0.90
Total	1,057	1,135	0.93
Control (Wounded)			
1919-1929	99	155	0.64
1930-1939	169	180	0.94
1940-1949	305	307	0.99
1950-1959	507	527	0.96
1960-1965	383	421	0.91
Total	1,463	1,590	0.92

---

[a] Data from Norman.[56] Expected deaths based on U.S. white age-specific male mortality data for 1919-1965.

was greater among men exposed to mustard gas than among the pneumonia or wounded cohorts (Table 4-8). These data suggest that gassed men experienced a 40% excess of lung-cancer mortality. The risk relative to controls, 1.4 (95% confidence limits, 0.9-1.9), was less than that required for statistical significance. Beebe and Norman discussed the possible influence of cigarette-smoking as an additional risk of lung cancer. Statistically, the differences were insignificant. However, direct observations on smoking were not generally available. Furthermore mortality among men in the mustard roster was generally greater than that in the other rosters--except for 1959-1960, but these exceptions were not great.

Although Case and Lea[13] concluded that exposure to H tended to increase the development of bronchitis and might therefore be a cause of respiratory cancer, even indirectly, Beebe[8] and Norman[56] failed to find a significant association between bronchitis and cancer. In contrasting the lack of a statistically significant proof of military H exposure as a cause of respiratory cancer with the highly significant excess of lung-cancer mortality in Japanese H factory workers, Norman suggested that the usual single exposure in military service was insufficient to be carcinogenic. The carcinogenicity of prolonged exposure to H was convincingly shown by the Japanese data.

Experimental and epidemiologic support for the carcinogenic potential of mustard gas has been confirmed by a World Health Organization review of data from all sources and periods of time and not merely World War I.[36]

EFFECTS ON HUMAN SUBJECTS AT EDGEWOOD

Between 1955 and 1965, 147 human subjects underwent exposure to H at Edgewood. One hundred sixteen masked subjects had aerosol chamber exposures to test the effectiveness of various protective garments. Equipment was tested for leaks with chloropicrin exposures before H exposures. Subjects underwent up to 14 exposures to H on different days and were removed from the tests when dermal erythema indicated garment leakage. Some tests simulated tropical or windy conditions, and others simulated battlefield functions. Thirty-one subjects had cutaneous exposures to test the effectiveness of antidotes or treated cloths or for sensitization.

In 1955, 11 subjects underwent up to 10 aerosol exposures lasting 17-22 min each. The maximal cumulative Ct was 6,000 mg min/m^3. Five subjects sustained dermal effects and discontinued the test after three to eight exposures. Erythema occurred on the upper chest and flexor surfaces of the arms. Eight subjects had normal post-exposure blood counts and urinalyses.[44]

TABLE 4-8

Observed and Expected Deaths Due to Respiratory Cancer, by Age at Death and by Roster, 1919-1965[a]

Age at Death, Yr	No. Deaths							
	Mustard Gas		Pneumonia		Wounded		Total	
	Observed	Expected	Observed	Expected	Observed	Expected	Observed	Expected
26-29	0	0.03	1	0.02	0	0.03	1	0.08
30-34	0	0.13	0	0.09	0	0.12	0	0.34
35-39	0	0.27	0	0.19	0	0.26	0	0.72
40-44	1	0.66	0	0.45	0	0.64	1	1.75
45-49	5	1.85	0	1.28	2	1.80	7	4.93
50-54	3	3.80	5	2.66	1	3.73	9	10.19
55-59	13	7.69	5	5.36	12	7.55	30	20.60
60-64	15	12.46	3	8.51	14	12.07	32	33.04
65-69	14	16.47	10	11.44	13	16.11	37	44.02
70-74	14	13.71	8	9.92	5	13.86	27	37.49
Total	65	57.07	32	39.92	47	56.17	144	153.16

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[a] Data from Norman.[56] Expected deaths based on U.S white age-specific male mortality data for 1919-1965.

In 1955, 12 subjects underwent up to 14 aerosol exposures of 45 min each. The maximal cumulative Ct was 30,800 mg·min/m^3. Ten subjects sustained dermal effects and discontinued the test after eight to twelve exposures. Erythema occurred on trunks and extremities. Three subjects had blisters. One subject, whose total Ct was 17,700 mg·min/m^3, was hospitalized 5 d after his last exposure because of diffuse erythema and bullae.[44]

In 1957, two subjects had three daily aerosol exposures of 499, 1,000, and 2,031 mg·min/m^3. No leaks occurred.

In 1963, 18 subjects underwent up to 10 exposures of 14-60 min each. The maximal cumulative Ct was 9,504 mg·min/m^3. Five subjects sustained dermal effects and discontinued the tests after six to eight exposures. Erythema occurred on backs and extremities. One subject developed marked vesication of his extremities 7 d after the last exposure. His total Ct was 5,898 mg·min/m^3.[57]

In another 1963 experiment, 13 subjects underwent up to 10 aerosol exposures of 15-60 min each. The maximal cumulative Ct was 16,000 mg·min/m^3. There were no injuries.

In 1964, 12 subjects underwent up to 10 aerosol exposures of 15-60 min each. The maximal cumulative Ct was 10,800 mg·min/m^3. Eight subjects had dermal effects, and three discontinued the test after six to eight exposures. Erythema occurred on backs and flexor surfaces of the extremities.[52]

In another 1964 experiment, 10 subjects had two applications of 4 mg of H on their forearms. Each drop was treated with an experimental decontaminant (Decon 10 and M-5 ointment). All exposure sites developed erythema and five vesicated.

In another 1964 experiment, 12 subjects underwent up to 10 aerosol exposures of 15-60 min each. The maximal cumulative Ct was 10,400 mg·min/m^3. Nine subjects developed dermal effects, and seven discontinued tests after four to eight exposures. Erythema occurred on anterior trunks, genitalia, and groin. One subject developed a vesicle.[58]

In another 1964 experiment, 10 subjects had H at 2 g/10 cm^2 on three separate swatches of protective cloth taped to their arms. The protocol notes that the cloth was protective in nine subjects. One subject was hospitalized when the exposed area vesicated with later eschar formation.

In 1965, 36 subjects underwent up to 10 exposures of 15-60 min each. The maximal cumulative Ct was 10,300 mg·min/m^3. Thirty-four subjects had dermal effects and discontinued the tests after two to nine exposures. There was mild to moderate erythema on the necks.[59]

In another 1965 experiment, 11 subjects had percutaneous H exposures to test for skin sensitivity. Ten of these subjects had previous aerosol H exposures. Each subject had seven daily exposures of moving H vapor at the same site for a total Ct of 1,100 mg·min/m^3. There were then three daily exposures, at a different site, to static H vapor with a Ct of 200 mg·min/m^3 each day. Two subjects had mild erythema on the latter sites at the completion of the tests.

In 1966, 10 subjects underwent up to 10 aerosol exposures of 15-60 min each. The maximal cumulative Ct was 10,250 mg·min/m^3. Four subjects developed burns, and two discontinued tests after 6 and 7 d. Erythema occurred on necks.[39]

In summary, the records of the 147 men who participated in tests involving H were reviewed. Many subjects sustained dermal injuries. No reactions to exposures were observed in 59 men. An additional 77 men experienced erythema without skin blistering. Blistering was seen in 11 men, two of whom were hospitalized, one for 5 d. None of these subjects sustained ocular or respiratory tract injury. This indicates that the ocular and respiratory systems were adequately protected during these tests. A few of the skin injuries might have been severe enough to cause permanent scarring. Although subjects entered the H vapor several times, they were protected by special garments, and exposures were discontinued after dermal injuries indicated equipment failure (leaks). Subjects' actual exposures to H were therefore limited, and there was no evidence of acute pulmonary or ocular injuries. Given the absence of followup data, it is not possible to predict long-term health effects, except scarring from acute injuries.

SUMMARY

Mustard gas is mutagenic in various organisms and test systems. One cannot readily predict the degree of genetic risk that it poses for man, however, because data on its mutagenicity in mammalian germ cells are very limited, and the mutagenic potency of mustards varies considerably among assay systems. Nevertheless, the available evidence suggests that the possibility of mutagenic effects of mustard gas in human germ cells should not be disregarded. The clear mutagenicity of mustard gas in various assays is consistent with its carcinogenic potential.

Mustard gas is not only a vesicant, but also a systemic poison. Its acute effects have been demonstrated in bone marrow, intestinal tract, and respiratory tract. It can cause blindness and permanent skin scarring with a potential for skin tumors. It probably can also cause acute and chronic bronchitis. Other nonmalignant chronic effects have not been adequately documented.

Single exposures, even if severe, as in military service, are not associated with statistically verifiable increases in mortality from tuberculosis and cancer; but repeated small exposures, such as occur in industrial operations, do increase cancer deaths significantly.

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o-CHLOROBENZYLIDENE MALONONITRILE

CHARACTERISTICS

o-Chlorobenzylidene malononitrile (CS)--also called *o*-chlorobenzalmalononitrile and *o*-chlorobenzylidine malononitrile (see Table 4-10) is a white, crystalline solid that melts at 95°C, boils at 310-315°C, and has a faint, peppery odor. It is almost insoluble in water, but breaks down rapidly in water and body fluids, forming less active compounds. At a pH of 7.4 in water, the half-time for breakdown is 14 min.[3] CS is moderately soluble in alcohol and soluble in acetone, dioxane, methylene dichloride, ethyl acetate, and benzene.[32] CS, first prepared by Corson and Stoughton[12] in 1928, is a strong sensory irritant. When it came into use in the 1960s as a riot-control agent, the long chemical name was replaced with the initials of the chemists.

CS is a lacrimator that is more effective, less toxic, and 10 times more potent than CN.[3,42] The vapors of CS are extremely irritating to the eyes and respiratory tract. For these reasons, it has largely replaced Mace (CN) as a riot-control agent. It is disseminated by spraying in powder form, sometimes mixed with an anticaking silicate (Cab-o-Sil), spraying in a liquid carrier, or incorporating in various types of grenades as a pyrotechnic mixture.

BIOCHEMISTRY AND PHYSIOLOGY

CS causes alkylation of sulfhydryl-containing enzymes and inhibits lactic dehydrogenase, glutamic dehydrogenase, pyruvic decarboxylase, and alpha-glycerophosphate dehydrogenase.[24,40] It reacts with a number of nucleophilic compounds, such as glutathione, plasma protein, and lipoic acid.[24]

Estimates of the incapacitating and lethal concentration-time products (ICt_{50} and LCt_{50}) of CS in man are 0.1-10.0 and 52,000-61,000 mg·min/m^3, respectively.[28] As an aerosol, it has a toxic concentration (TC_{50}) for the eye of 4 x 10^{-3} mg/m^3 and an IC_{50} of 3.6 mg/m^3.[3] A concentration of 4 mg/m^3 is effective in riot-control conditions for harassment,[3,20] but 10 mg/m^3 may be used in field conditions to disperse troops.[20] Small particles of CS are more effective than large particles to produce rapid ocular and respiratory irritation, as well as rapid recovery after exposure.[3,16] The minimal detectable irritant concentration of CS is 0.004 mg/m^3,[20] and lacrimation occurs at 1 mg/m^3.[49] Although the human lethal dose of CS by inhalation is estimated as 25,000-150,000 mg·min/m^3, no deaths from use of CS aerosols have been documented.[3]

Cucinell _et al_.[13] reversed the CS inhibition of lactic dehydrogenase in dogs by injecting sodium thiosulfate. Rats poisoned by CS at 80 mg/kg (more than the LD_{50}) were saved by injections of thiosulfate. Cucinell _et al_. speculated that the toxic effects of CS were caused by inhibition of sulfhydryl-containing enzymes.

Cucinell _et al_. examined some physiologic reactions to CS in the dog. A spray containing CS at 25 μg/L caused an increase in blood pressure, tachycardia, and changes in respiratory pattern. The dogs yelped and might have been in pain. Release of bradykinin in rabbits tested with CS may have been related to the pain caused by CS.[13]

TOXICOLOGY IN IN VITRO AND ANIMAL STUDIES

Mutagenicity

CS has been tested for its capacity to induce mutations in bacteria and in the fruit fly _Drosophila melanogaster_ and for its capacity to cause chromosomal damage, as measured by a micronucleus test, in mice. Its capacity to bind to DNA in mammalian liver and kidney has also been studied. The results have been predominantly negative.

Von Däniken _et al_.[47] tested CS for mutagenicity in the Salmonella/microsome test in the standard tester strains TA1535, TA1537, TA1538, TA98, and TA100 of _Salmonella typhimurium_. Tests were conducted both with and without an in vitro metabolic activation system and were performed both by the standard _Salmonella_/microsome plate-test procedure and by a preincubation modification of that procedure. Only strain TA100 showed evidence of mutagenicity, and its response was weak. The maximal increase in numbers of revertants per plate was by a factor of about 2, and that increase occurred at the high chemical concentration of 1 mg/plate.

In two recent studies of CS, the weak mutagenicity reported by von Däniken _et al_.[47] has not been confirmed. Rietveld _et al_.[37] (1983) described CS as nonmutagenic in _S. typhimurium_ strain TA100 both in the presence and in the absence of a rat liver metabolic activation system. The compound was tested at concentrations up to 1 mg/plate; at higher concentrations, bacterial toxicity was observed. Unfortunately, no data were presented to support the conclusion of nonmutagenicity, and an independent evaluation of the results is therefore not possible.

Wild *et al*.[48] also reported CS to be nonmutagenic in the *Salmonella*/microsome test. Tests were conducted in strains TA1535, TA1537, TA1538, TA98, and TA100 both with and without a rat liver metabolic activation system. Concentrations up to 1.5 mg/plate were tested, and toxicity was noted at the high concentrations. Data were presented only on strain TA100 in the absence of metabolic activation; the data on a negative control, a positive control, and eight concentrations of CS supported the authors' conclusion of nonmutagenicity. Although the result would be more convincing if data were available on all strains and on tests with metabolic activation, it is noteworthy that the negative data presented were on the same strain in which von Däniken *et al*. reported a weak positive response.

Von Däniken *et al*.[47] (1981) also tested CS for its ability to bind to DNA and protein in rat liver and kidney. When CS labeled with ^{14}C was administered to rats by intraperitoneal injection, it bound readily to liver and kidney nuclear protein, but not to DNA in these organs. From these results, von Daniken *et al*. concluded that mutagenesis and carcinogenesis from CS exposures would be unlikely in humans. It should be noted that binding studies do not measure defined genetic events and therefore do not provide a strong basis for regarding a substance as nonmutagenic. Because other tests for genetic damage have been negative, however, the failure to detect binding to DNA can be regarded as consistent with the negative results.

In a test for sex-linked recessive lethal mutations in *Drosophila*, Wild *et al*.[48] found no evidence of mutagenicity of CS. More than 9,000 chromosomes were tested, and the frequencies of mutations in the treated groups did not differ from those in the concurrent negative controls or the historical negative controls. The available information on the toxicity of CS under the treatment conditions is minimal. The actual dosages received by the flies are also uncertain, particularly because CS breaks down rapidly in water. Nevertheless, the available data give no indication of mutagenicity of CS in *Drosophila*.

CS has been tested for its ability to cause chromosomal damage in a micronucleus test in mice. Micronucleus tests detect small nuclei that arise from chromosomal fragments or chromosomes that fail to be incorporated into normal daughter nuclei when cells divide. An increased frequency of micronuclei is evidence of chromosomal breakage or loss. No increase in the frequency of micronuclei was observed in polychromatic erythrocytes in the bone marrow of mice treated with CS. Treatments were given either by oral administration or by intraperitoneal injection, and they included appropriately toxic dosages.

Taken in their totality, the tests of CS for gene mutations and chromosomal damage in several organisms provide no clear evidence of mutagenicity. In fact, most of the evidence is consistent with nonmutagenicity. The available data are not sufficient to preclude mutagenicity absolutely. However, in the Committee's judgment, it is unlikely that CS poses a mutagenic hazard to humans.

Teratogenicity

In 1973, Upshall[46] reported tests of CS for teratogenicity in female Porton strain rats and New Zealand White rabbits. He attempted to simulate conditions that exist in riot-control situations, looking for teratogenesis and changes in numbers of offspring in response to aerosol exposure of both species. Control rats were subjected to handling stress and aerosols without CS.

Aerosol exposures were at 6, 20, and 60 mg/m^3 for 5 min on days 6-15 of pregnancy in rats and days 6-18 of pregnancy in rabbits. Control aerosols consisted of water or suspensions of Neosil (silica dust), and the experiments were conducted with 12-24 animals. The adults were killed a day before parturition; rat fetuses were examined for 18 abnormalities and rabbit fetuses for eight abnormalities. In addition, rats were studied for teratogenic effects after exposure to CS by intraperitoneal injection at 20 mg/kg on day 6, 8, 10, 12, or 14 of pregnancy.

The results led Upshall to conclude that CS is neither teratogenic nor lethal to embryos. No significant increases in numbers of abnormal fetuses or in resorptions were noted. No dose-related effects were observed, except in one experiment in which rats had marginally lower fetal weights. A high incidence of abnormalities occurred in two control groups of rats.

There is no evidence of developmental toxicity of CS in the Upshall study, but the exposure conditions were limited. The short (5 min/d) exposures and seemingly low dosages did not fully test the potential teratogenicity of CS. In riot-control situations, humans may be exposed to CS at 4-10 mg/m^3,[46] and the absolute concentrations used in this study were 6, 20, and 60 mg/m^3. Without considering time of exposure, the sixfold difference in concentrations (60 vs. 10 mg/m^3) between humans and test animals may not constitute a sufficient margin for an adequate assessment of teratogenic risk. No data were presented on maternal systemic toxicity or mortality at those dosages. Teratology studies are routinely performed at dosages up to those which cause maternal mortality, so that the relative sensitivity of the pups and mothers can be compared. A possible source of misinterpretation in the study is that the exposures appear to have been whole-body exposures, rather than "nose-only"; this means that an animal could bury its eyes and nose in its fur to minimize irritation and thereby lower the effective exposure during the 5-min exposure period. Although those problems do not apply to the injection study, the lack of toxicity data makes it impossible to conclude definitively that CS would not be teratogenic under other exposuure conditions. On balance, one can conclude only that under the conditions of this study CS did not exhibit teratogenic or fetotoxic activity.

Carcinogenicity

The National Toxicology Program in the United States performed a subchronic study of CS to generate data on the maximally tolerated dose (MTD) of this agent preparatory to launching a full-scale chronic toxicity and carcinogenicity bioassay.[45] These tests were conducted in Fischer 344 male and female rats and in $B6C3F_1$ hybrid male and female mice. Six groups of 20 rats and mice divided equally between sexes were exposed to an aerosol of CS by inhalation at concentrations of 0 (control), 0.40, 0.75, 1.5, 3.0, and 6.0 mg/m^3 for 6 h/d, 5 d/wk, for 13 wk, for a total of 65 exposures of 6 h each. In both species, exposures at the two highest doses (3.0 and 6.0 mg/m^3) led to listlessness and mouth breathing. The clinical signs included a hunched appearance, squinting, and closure of eyes at all concentrations of CS. However, at the end of 13 wk, there were no gross lesions that could be definitely attributed to exposure to CS. Microscopically, in both species there was extensive inflammation with erosions of the nasal epithelium in animals exposed at 0.75 mg/m^3 or higher. Rats exposed at 1.5 mg/m^3 or higher had epithelial hyperplasia and squamous metaplasia of the nasolacrimal duct and squamous metaplasia of the trachea and larynx. Females exhibited the same picture as males, but somewhat less severe. On the basis of the combined data, it was recommended that the chronic study could be conducted at 1.5 mg/m^3.

McNamara _et al._[29] tested CS for carcinogenicity in mice and rats. Groups of 100 (50 male and 50 female) A/J strain (tumor-sensitive) mice and Sprague-Dawley-Wistar rats were exposed to CS at high and low doses in methylene chloride, to methylene chloride alone, and to urethane in methylene chloride. Controls were unexposed mice and rats in the same numbers. During the 24-mo experiment, groups of animals were killed and examined for tumors (Table 4-9). All groups gained weight with no significant differences for the first 12 wk, after which weights were not checked. During the exposure period (5 d/wk for 4 wk), one control and 14 experimental mice died; one control rat and 10 exposed rats died. Urethane was used as a positive control, because it induces tumors under some conditions. In this experiment, urethane caused a significantly greater incidence of tumors than the methylene chloride controls (38% vs. 22%). No tumorigenic effects were found in the CS-exposed animals.

There were no statistically significant differences among the other groups. It was concluded that, because the high Ct's used were not significantly tumorigenic, the Ct's to be expected in a riot-control situation (1.0-10.0 mg·min/m^3) would not be dangerous. Data on small-animal toxicity have been collected by Ballantyne[3] (Table 4-10).

Lethal Effects

The Himsworth report[19,20] recommended that any chemical agent that might be used as a riot-control agent be studied in the same way as a new therapeutic drug. To a considerable extent, this has been done with CS.

Cucinell *et al.*[13] reported an LCt_{50} of 57,000 mg·min/m^3 for the anesthetized dog. McNamara *et al.*[28] compared LCt_{50} values for six animal species exposed to aerosols of CS generated by melting and spraying the agent with smokes from M7A3 grenades (Table 4-11).

Striker[43] exposed 32 immature *Macaca mulatta* monkeys to CS aerosols in a chamber at four concentrations in groups of eight (Tables 4-12 and 4-13). At 80,000 mg·min/m^3, the highest [illegible] five monkeys died soon after exposure. The others, including controls, were killed for examination at 12 h, 72 h, 1 wk, and 30 d. Only mild symptoms of pulmonary congestion were seen at Ct's of 2,700 and 8,500 mg·min/m^3. At a Ct of 28,500 mg·min/m^3, severe symptoms developed and pneumonia occurred. At a Ct of 80,000, mg·min/m^3, three survivors had important lesions (edema, emphysema, and bronchiolitis) at autopsy. Striker[44] similarly exposed monkeys to lower Ct's, with five animals at each (Table 4-13). Only mild coughing and nasal discharges were observed after these exposures. No lesions were found on necropsy. They concluded that no cumulative or systemic injuries follow long exposures at low concentrations.

TABLE 4-9

Pulmonary Tumors in Animals after 20 Inhalation Exposures to Various Chemicals[a]

Chemical	Average Daily Ct, mg·min/m^3	No. Animals with Pulmonary Tumors/No. Animals Tested									
		A/J Mice					SDW Rats				
		6 mo	12 mo	18 mo[b]	24 mo	Total	6 mo	12 mo	18 mo[c]	24 mo	Total
Controls	--	2/24	0/25	15/25	2/7	19/81	0/26	0/23	1/25	0/12	1/86
Methylene chloride	500	2/24	2/23	4/25	5/12	13/84	0/24	0/26	0/25	0/20	0/95
Urethane	500	5/24	7/25	16/29	3/3	31/81	0/23	2/25	0/26	0/17	2/91
CS	50	0/24	3/25	14/25	7/8	24/82	0/23	0/24	0/25	0/17	0/89
CS	500	2/22	5/25	11/25	0/4	18/76	0/25	1/29	2/25	0/16	3/95

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[a] Data from McNamara[9]

[b] Subcutaneous tumors: 14 of various types; 7 in controls, none in CS-exposed mice.
Internal organ tumors: 1 uterine tumor in a CS-exposed rat.
4 tumors in controls: 2 in liver, 1 in kidney, 1 in spleen.

[c] Other tumors: 16 mammary tumors: 2 adenomas, 10 fibroadenomas, 3 adenocarcinomas, 1 fibroma. In skin: 1 inclusion cyst.

[d] One rat had a fibroadenoma in the inguinal region.

[e] One rat had an ovarian granulosa cell tumor.

TABLE 4-10

Comparative Acute Mammalian Toxicity of 10-Chloro-5,10-dihydrophenarsazine (DM), Chloroacetophenone (CN), 2-Chlorobenzylidene Malononitrile (CS), and Dibenz[b,f][1,4]oxazepine (CR)[a]

		LD_{50}, mg/kg, or LCt_{50}, mg·min/m^3 [c]			
Route	Species	DM	CN	CS	CR
Intravenous	Rabbit	6-26	20-31	23-27	47
	Rat	26	35-41	28-35	68
Intraperitoneal	Rat	164	36-56	48-66	766-817
Oral	Rat	563	52-258	178-1,366	
	Rabbit		118	142-401	1,760
Inhalation[b] (pure material)	Rat	3,700-12,710	3,700-18,800	88,480	425,000
	Mouse	22,400-46,245	18,200-73,500	67,200	169,500
	Guinea pig	6,599-7,900	3,500-13,140	50,000	169,500
	Rabbit		5,842-11,480	54,100	169,500
Inhalation[b] (smoke)	Rat	48,217	23,330	68,000	139,000
	Mouse			76,000	203,000
	Guinea pig	29,888	15,400	35,000	169,000
	Rabbit	46,959	15,800	63,000	169,000

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[a] Derived from Ballantyne.[3] Ranges cover results from various authors.

[b] LCt_{50}, values for exposures at high concentration for short duration.

[c] LCt_{50} for inhalation, LD_{50} for other routes.

TABLE 4-11

Lethal Effects of CS in Animals Exposed by Inhalation[a]

	Molten CS		M7A3 Grenade	
Species	No. Animals	LCt_{50}, mg·min/m^3	No. Animals	LCt_{50}, mg·min/m^3
Mouse	120	41,790	--	--
Rat	70	32,293	160	94,378
Guinea pig	70	8,410	220	65,573
Rabbit	20	17,452	66	37,683
Dog	36	33,551	42	29,748
Monkey	31	50,089	30	123,195

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[a] Data from McNamara *et al.*[28]

TABLE 4-12

Exposures of Monkeys to CS at High Ct's[a]

Duration of Exposure, min	Average Concentration, mg/m^3	Ct, mg·min/m^3
3	900	2,700
5	1,700	8,500
10	2,850	28,500
32	2,500	80,000

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[a] Data from Striker.[43]

TABLE 4-13

Exposures of Monkeys to CS at Low Ct's[a]

Duration of Exposure, min	Average Concentration, mg/m^3	Ct, mg·min/m^3
5	53	265
10	55	550
30	50	1,500
5	305	1,525
10	307	3,070
30	304	9,120

---

[a] Data from Striker.[44]

Ballantyne and Callaway[4] exposed four species of animals to CS smoke generated from a standard grenade. In the first experiment, groups of animals were exposed for 5, 10, 15, and 20 min at a mean concentration of 4 g/m^3. LCt$_{50}$ values were estimated as follows:

	LCt$_{50}$, mg·min/m^3	
Species	Mean	Range
Guinea pig	35 x 10^3	25-45 x 10^3
Rabbit	63 x 10^3	50-80 x 10^3
Rat	68 x 10^3	61-77 x 10^3
Mouse	76 x 10^3	61-119 x 10^3

The experiment was repeated, but with a mean CS concentration of about 38.3 mg/m^3 and longer exposure times (5-35 h). The corresponding LCt$_{50}$ values were as follows:

	LCt$_{50}$, mg·min/m^3	
Species	Mean	Range
Guinea pig	48.6 x 10^3	22.4-98.4 x 10^3
Rabbit	54.1 x 10^3	18.8-243.0 x 10^3
Rat	25.2 x 10^3	8.7-52.9 x 10^3
Mouse	36.1 x 10^3	20.9-61.0 x 10^3

No animals died during exposure. Deaths occurred later, and examination revealed mainly damage to the pulmonary system. Survivors showed no residual pathologic effects when sacrificed and examined 14 d later. The authors commented that, although guinea pigs and rabbits seem to be equally susceptible to CS under both sets of conditions, rats and mice are more susceptible to long exposures at low concentrations.

A second series of experiments compared rats and hamsters under three exposure conditions: 150 mg/m^3 for 2 h (Ct, 18,000 mg·min/m^3), 480 mg/m^3 for 1 h (Ct, 28,800 mg·min/m^3), and 750 mg/m^3 for 30 min (Ct, 22,500 mg·min/m^3). At the lowest Ct, two animals died, but survivors had only negligible effects. At a Ct of 22,500, there were no deaths; five survivors had minor lesions in lungs and kidneys. At a Ct of 28,800, 40 deaths were caused by extensive damage to lungs and kidneys; among survivors, 13 of 70 had minor lesions in lungs, kidneys, and liver.

In a third series, 56 rats were exposed to pure CS at 1-2 g/m^3 for 5 min on 5 successive days. Another 50 rats were exposed to CS at 12-15 mg/m^3, 80 min/d for 9 successive days. There were no deaths among animals exposed at the high concentration; a few survivors had minor lesions. Five rats examined 2-3 wk later had developed bronchopneumonia. There were five deaths from bronchopneumonia among the low-concentration animals and nine similar infections among the survivors. It was concluded that animals exposed repeatedly to CS may become susceptible to pulmonary infections.

Care must be taken in comparing LCt_{50} values, as was shown by Ballantyne and Swanston,[8] who described toxicities of CS and CN given by various routes. The CS was pure, thermally generated, rather than from grenades. They measured LCt_{50} values for the inhaled agent. In comparison with the values of the first experiment of Ballantyne and Callaway (above), they estimated the following:

Species	Mean LCt_{50}, $mg \cdot min/m^3$
Guinea pig	67.2×10^3
Rabbit	54.1×10^3
Rat	88.5×10^3
Mouse	50.0×10^3

The chief variable appears to have been the change from grenade-generated CS to pure CS, but there were also differences in exposure times and concentrations. It is assumed that the general procedures and equipment were the same.

Ocular Effects

Owens and Punte[30] tested CS solutions in rabbits and monkeys by dropping 0.2 ml of 1% CS in dipropylene glycol into one eye of each rabbit or monkey, the other eye serving as a control. Six animals were used in each test. Conjunctival redness and swelling lasted for 1-3 d. The experiment was repeated, but with applications of 0.2 and 0.05 ml on 5 successive days. The 0.2-ml applications produced conjunctivitis, iritis, severe chemosis, and corneal ulceration. The 0.05-ml applications resulted in conjunctivitis, moderate chemosis, and iritis. These conditions had all cleared 7-10 d after the last dose. Rabbits and monkeys had qualitatively similar symptoms, but they were less severe in the monkeys.

Aqueous sprays of 0.1 and 0.25% CS into the human eye caused no histologic ocular changes.[35] Spray of 0.5% into the rabbit eye caused transitory corneal changes.[36]

Ballantyne et al.[6] examined the ocular effects of CS in polyethylene glycol (PEG) solutions, as a powder, and as smokes. Test solutions were made up in concentrations of 0.5, 1, 2, 5, and 10% CS in PEG. Each was tested on 10 rabbits with 0.1-ml drops instilled into one eye. Four groups of 20 rabbits were tested at 0.5, 1, 2, and 5 mg of CS as a powder. Ten rabbits were exposed to CS grenade smoke for 15 min; the average CS concentration was 6 g/m^3. The effects of the hydrolysis products of CS were also examined. Ten animals were tested with o-chlorobenzaldehyde and 10 with malononitrile. The amounts of the two compounds were determined as the ratio obtained by the hydrolysis of a 5% solution of CS. This was a

3.72% solution of *o*-chlorobenzaldehyde and a 1.75% solution of malononitrile. Again, 0.1 ml was instilled into a rabbit eye.

The effects of the CS solutions ranged from mild, transient lacrimation, blepharitis, chemosis, and congestion at 0.5%, which cleared in 24 h, to iritis lasting up to 7 d and keratitis lasting over 45 d, at 10%. Powdered CS had milder effects than solutions. Lacrimation was mild and lasted 24 h at all doses. Blepharitis ranged from just detectable to mild as the amount of CS increased; it cleared in a week. A just-detectable iritis and keratitis lasting 24 h occurred in two of 10 rabbits given 5 mg; all showed mild chemosis. Exposure to 6-g/m^3 smoke for 15 min caused transient lacrimation and just-noticeable blepharitis lasting 24 h. The breakdown products of CS, which were estimated to be equivalent to the hydrolysis of a 5% solution of CS, caused mild lacrimation, blepharitis, and chemosis of short duration.

The authors concluded that direct application of CS-PEG solutions over the surface of the eye caused increased absorption. Ocular contact with CS powder or smoke may have permitted removal of the agent by excessive lacrimation. Damage to the eye can occur with CS solutions of 1% or more. Smokes and powders do little harm, even in relatively high amounts.

Rengstorff[33] measured the effects of CS in a wind tunnel on visual acuity of 10 young human volunteers with 20-20 vision--six with CS at 0.1 min/m^3, one at 1.3 $mg \cdot min/m^3$, two at 1.6 $mg \cdot min/m^3$, and one at 1.7 $mg.min/m^3$. Rengstorff also exposed 34 men to CS in a chamber, seven at 0.4, 17 at 0.6, six at 0.9, and four at 1.0 $mg \cdot min/m^3$. The men were free to leave the chamber at will; a few left in less than 1 min, but half stayed for a full 10 min. Rengstorff and Mershon[35] tested 10 men by instilling one drop of 0.1% CS (five men) or 0.25% CS (five men). The water contained 0.5% polysorbate 20 as a carrier. A second group of men received the same CS mixture as a 2-s spray. Four men received the 0.1% CS and two the 0.25% CS. Rengstorff and Mershon[34] studied the safety of trioctyl phosphate (TOF) as a vehicle for CS. As in the preceding experiment, some men received CS in TOF by the instillation of a drop into one eye; two men each were tested with 0, 0.05, 0.1, 0.25, 0.5, and 1.0% CS. Eight other men were sprayed in one eye with various concentrations of CS: two at 0.1%, one at 0.25%, one at 0.5%, and four at 1.0%.

In all these tests, the men were asked to read Snellen chart numbers as soon as possible after opening their eyes. In the chamber test with CS aerosols, an Orthorater (Bausch and Lomb vision tester) was used to measure near and far visual acuity. All the treated eyes were then examined by ultraviolet and slitlamp procedures to detect corneal injury. The results of all three experiments may be summarized as follows: There were fairly wide variations in the times after exposure to eye-opening, but normal vision was restored in all subjects a few minutes after the eyes were opened. Postexposure corneal inspections revealed no injuries either immediately or later. CS aerosols, CS in water with polysorbate 20, and CS in TOF presented no harmful effects on visual acuity.

Ballantyne and Swanston[9] developed a laboratory procedure to measure the threshold concentrations of CS that produced sensation in the human eye and tongue, to compare various irritant agents. Threshold concentrations of CS were also measured in the rabbit and guinea pig with blepharospasm as the criterion of ocular response. CS at various concentrations was dispensed into the eyes and onto the tongue in 0.01-ml droplets. The effective concentrations for 50% of the subjects (EC_{50}s) for blepharospasm were as follows: guinea pig, 2.2×10^{-5} M; rabbit, 5.9×10^{-5} M; and man, 3.2×10^{-6} M. The human eye (just-noticeable) sensation threshold was 7.3×10^{-7} M (0.14 mg/L), and that for the tongue, 6.8×10^{-6} M.

On the basis of comparison of their figure for CS in solution with an EC_{50} for CS aerosols (unpublished) of 4.0×10^{-3} mg/m^3 of air, the human eye was much more sensitive to CS aerosol than to CS in solution. This can be explained by the great dilution of molecular CS in solution compared with the concentrated action on a sensory nerve ending of a micrometer-sized particle in an aerosol. Humans are more sensitive than the test animals, so caution is required in extrapolating animal data to humans. The authors estimated a 6,650-fold safety factor between the EC_{50} for these threshold values and the CS concentration likely to cause the least detectable corneal damage in the human eye.

Cutaneous Effects

Single applications of 1.0 ml of 1% CS solution were administered to the clipped backs of rabbits.[29] They developed mild to moderate erythema that cleared in 3 d. Monkeys showed no signs of irritation. A 5-d test of the 1% solution caused only moderate erythema that cleared in 8 d. A 0.2-ml application caused only moderate irritation in the rabbits. In monkeys, 1-ml and 0.2-ml applications caused no irritation. Patch tests on rabbits and monkeys with similar doses caused somewhat more skin irritation, but generally the lesions were reversible; no systemic toxicity was demonstrated.[30]

One study[7] prompted by the Himsworth report examined the extent of burns inflicted by pellets from CS grenades and the possibility of their interference with the healing of wounds suffered by rioters. Anesthetized guinea pigs were subjected to light skin abrasions, deeper skin wounds, skin burns, and effects produced by allowing an ignited CS grenade pellet to burn on the skin (10-15 s). Controls were provided. Wounds and burns not inflicted by pellets were contaminated by CS, both dry and dissolved in diethyl ether. Healing of CS-contaminated animals and controls did not differ significantly.

Tests of CS-Loaded Pen Guns

Ayers and Stahl[1] studied the ballistics of pen guns loaded with CS. When the gun was fired from a distance of 7.5 cm, no damage to the skin or underlying tissues of rabbits occured. At a distance of 2.5 cm, contusions and small lacerations of the skin were observed. On direct contact, one test resulted in a fractured femur. In another, over the chest, the rabbit was killed. The wad in the cartridge was the cause of the damage.

In a second experiment, Ayers and Stahl[2] studied the effects of discharging a CS pen-gun cartridge into a rabbit eye at a distance of 20 cm. At that distance, the wad caused less damage than the blast and the particles of CS. Only three of 10 animals suffered severe eye lesions, which appeared to combine mechanical damage (lacerations) from the wad and conjunctivitis, intraocular hemorrhage, keratitis, and corneal edema probably from the blast and CS particles driven into the eye. The other animals showed only mild conjunctivitis, which cleared after 3 d.

TOXICOLOGY IN HUMANS

Immediate Effects

The effects of CS are immediate and self-limiting. Recovery usually occurs within 30 min after exposure ceases.[3] The effects include a burning, pricking, or peppery sensation in the eyes, nose, mouth, throat, and skin; lacrimation, rhinorrhea, and salivation; blepharospasm and injection of the conjunctivas and margins of the eyelids; photophobia lasting up to 1 h in 10% of subjects; tightness of the chest associated with gripping pain, breathholding, dyspnea, coughing, and sneezing; erythema and occasionally vesiculation of exposed skin; and nausea, vomiting, headache, and apprehension.[3,19,20,28] Moisture worsens the skin effects.[11,22]

Tolerance to CS may develop from repeated exposures at low concentrations,[20] but it is reduced by hyperventilation, as well as by increased environmental temperature and humidity.[3] Whole-body exposure to CS solutions may result in a transient increase in blood pressure, but not to the extent observed with CR.[3,20] CS is a hapten and may cause allergic contact dermatitis, with erythema, edema, and vesication, which is less severe than the effects of CN.[3]

The effects of CS on persons exposed in confined spaces are similar to those described above, and recovery occurs quickly after exposure ceases. During the Londonderry riots, exposure of a child in a bedroom resulted in crying, gasping for breath, pallor, and lacrimation, but the child recovered promptly.[19]

A causal relationship between exposure to CS and asthmatic attacks has not been established, but irritant CS smoke may predispose susceptible persons to asthmatic attacks.[3,19,20] Similarly, persons with pre-existing chronic bronchitis may have superimposed acute bronchitis or bronchopneumonia after exposure to CS.[3,20]

Experimental Human Exposures

In some experiments, volunteers were exposed to CS aerosols through gasmasks arranged for air-agent passage in and out. Typically, men were exposed in a large wind tunnel in which CS concentration, air speed, and temperature were controlled. Table 4-14 shows test conditions.

The estimation of the incapacitating dose for humans is usually for a 1-min period, so the ICt_{50} is the concentration at which half the exposed population is affected.[28] Typically, men leave the exposure with tears, nasal secretions, and saliva pouring out, and towels rather than handkerchiefs are needed to cope with the fluids. In 5-15 min, the irritation ceases. Punte et al.[32] noted that men with a history of sinusitis felt such relief that, after the effects of CS subsided, they asked to take part in further tests. One man claimed to have improved hearing after exposure to CS.

Determining the ICt_{50} for humans entails some difficulty due to differences in motivation and tolerance. The official ICt_{50} for humans was therefore estimated by the Research Laboratories at Edgewood as a range: 0.1-10.0 $mg \cdot min/m^3$. The LCt_{50} for humans was derived from extensive animal data and was expressed for two conditions:

	LCt_{50}, $mg \cdot min/m^3$	Safety Factor
Molten CS	52,000	5,200-520,000
M743 grenade	61,000	6,100-610,000

The safety factor is obtained from the ratio $LCt_{50}:ICt_{50}$.[27] Table 4-15 shows animal data on which these values were based.

TABLE 4-14

Comparative Effectiveness of CS in Men Exposed under Various Conditions (Individual Experiments)[a]

Study	Year	Dissemination Method	Exposure Conditions	Concn., mg/m^3	No. Men	ICt_{50}[b] mg/m^3	
1	1959	Sprayed acetone solutions	Wind tunnel--5 mph; group exposures--total body; no specific psychologic motivation techniques used--men briefed before exposure and asked to resist agent to best of their ability	5-442	78	3.0	4.7
2	1967	Sprayed acetone solutions	Wind tunnel--5 mph; individual exposures--head only; no motivation--as for study 1	0.03-8.0	35	0.1	0.07
3	1968	Sprayed acetone solutions	As for study 1	0.02-5.4	30	0.3	0.2
4	1968	CS-methylene dichloride solutions dropped into heated cup	20-m^3 chamber; group exposures--total body; no motivation--as for study 1	0.40-0.90	21	0.7	0.6
5	1968	CS-methylene dichloride solutions dropped into heated cup	20-m^3 chamber; group exposures--total body; subjects motivated in groups by psychologic techniques	0.50-28.0	130	12.4	6.9
6	1968	CS2[c] dry powder disseminated	As for study 1	0.3-6.7	30	1.4	0.5

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[a] Data from McNamara *et al.*[28]

[b] 60 s tolerance time; Bliss analysis on left, curvilinear regression line analysis on right.

[c] CS2 = 95% CS, 4.75% Cab-O-Sil, and 0.25% hexamethyldisilazane.

TABLE 4-15

Munition (M7A3) Inhalation Toxicity[a]

Species	Ct, mg.min/m³	Concen., mg/m³	Exposure Time, min	Mortality
Monkey	246,400	4,265	62	6/6
	149,425	3,558	42	4/6
	119,600	3,739	32	2/6
	62,400	1,950	32	1/6
	55,950	3,730	15	0/6
Dog	72,160	2,488	29	6/6
	62,400	1,950	32	6/6
	50,050	1,925	26	3/6
	33,760	1,688	20	2/6
	27,880	1,991	14	1/6
	12,975	2,595	5	3/6
	4,216	1,054	4	0/6
Swine	72,160	2,488	29	6/6
	67,300	1,819	37	6/6
	34,070	3,786	9	5/6
	21,660	3,094	7	5/5
	13,975	2,595	5	1/6
	4,216	1,054	4	0/6
Goat	82,930	2,592	32	6/6
	67,300	1,819	37	4/6
	62,400	1,950	32	4/6
	43,900	2,927	15	1/6
	34,070	3,786	9	2/6
	21,660	3,094	7	1/5
	17,400	2,486	7	0/6
Rabbit	100,950	2,148	47	6/6
	86,360	1,661	52	6/6
	80,260	2,508	32	6/6
	76,800	1,829	42	4/6
	62,400	1,950	32	6/6
	55,950	3,730	15	6/6
	52,080	3,472	15	6/6
	50,050	1,925	26	5/6
	34,070	3,786	9	0/6
	21,660	3,094	7	1/6
	4,216	1,054	4	0/6
Rat	165,000	3,173	52	20/20
	123,200	1,987	62	16/20
	100,950	2,148	47	10/20
	86,360	1,661	52	7/20
	79,250	991	80	7/20
	76,800	1,829	42	4/20
	62,400	1,950	32	3/20
	21,660	3,094	7	0/20

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[a] Data from McNamara *et al*.[28]

Punte et al.[32] exposed volunteers to aerosol particles of 0.5-1.0 µm. The windspeed was 5 mph. Figure 4-3 shows the variability in response times, especially at low concentrations. These experiments were continued with exposures at various temperatures, with exercise, and with repeated exposures and long low-concentration exposures to develop tolerance. High temperatures and humidity reduce the response time, as does exercise. After tolerance was developed, men given simple problems required more time to complete them, but accuracy was not impaired. Airway resistance did not increase during exposure to CS. One group exposed 10 times over 2 wk at up to 13 mg/m^3 had normal blood electrolytes. Only minor adverse effects were observed in 75 men exposed in these experiments.

Owens and Punte[30] tested six of 50 volunteer subjects best able to tolerate CS, to compare the effects of small and large CS particles on the eye and respiratory system. The mass-median diameter of small particles was 0.9 µm, and that of large particles, 60 µm. Tolerance was defined as the time that the subject could remain in a wind tunnel spray (2% CS in methylene dichloride). Recovery was the time after exposure when they could sort 24 playing cards from which the corner numbers were removed. Small particles produced eye and respiratory irritation more rapidly. Large particles had longer-lasting eye effects. Small particles had a predominantly respiratory effect, whereas large particles had predominantly ocular effects (Tables 4-16 and 4-17).

Gutentag et al.[17] showed that CS, either dry or in solution, produced erythema and vesicles in the skin of human subjects. Covering the CS-treated area increased severity, and airtight compresses were more damaging than gauze pads.

Hellreich et al.[18] studied simulated tropical exposure conditions. CS aerosols were generated in an aerosol chamber (97°F, 100% relative humidity, windspeed of 5 mph) so as to expose the right forearm and hand of each subject. As controls, the left arms were exposed similarly, but without CS. Four subjects were exposed in each group, as follows:

Group	Time, min	Avg. Concentration, mg/m^3	Ct, $mg \cdot min/m^3$
I	15	296	4,440
II	30	316	9,480
III	45	312	14,040
IV	60	295	17,700

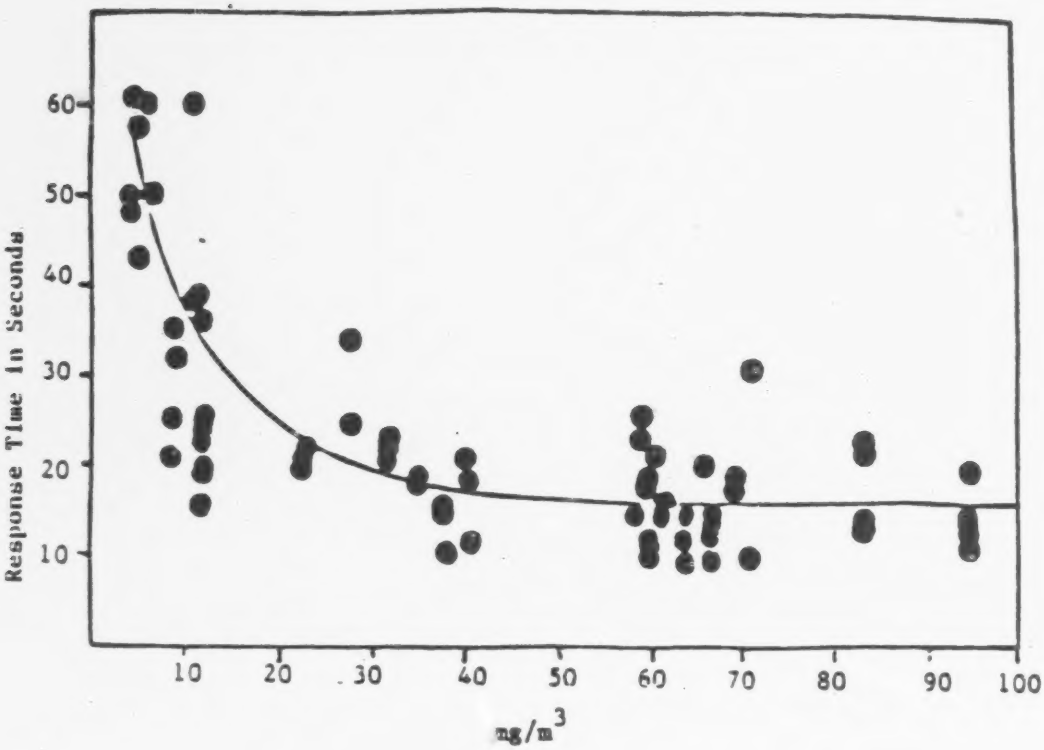

FIGURE 4-3 Response (tolerance time) of subjects exposed to CS. Reprinted with permission from Punte et al.[32]

Subjects in Groups I and II immediately developed erythema, which persisted up to 30 min after exposure and left no after-effects; all subjects reported a stinging sensation soon after the exposure began. In Groups III and IV, the immediate erythematous reaction was more severe, but subsided in 3 h; over the next 12-24 h, all subjects developed moderate to severe first-degree burns. One subject in Group III and three in Group IV progressed to second-degree burns. Medical care was provided, and the burns healed with no after-effects. (Effective medical care would not always be available in field situations.)

Holland and White[21] applied measured quantities of CS to the forearms of subjects under 4-cm-diameter sealed glass covers. The dry powder produced erythema in 30 min when 20 mg or more was used. When 2 drops of saline solution was added, the amount required was 10 mg. Erythema was transient, fading out in 1 or 2 days with no after-effects.

The indiscriminate effects of CS smokes in riot-control use prompted consideration of whole-body CS water spray as a more accurate way of delivering CS to desired targets.[5] Subjects were drenched in cubicles like shower stalls, receiving 15 L of spray in 15 s, with CS concentrations of 0.001, 0.002, 0.003, and 0.005%. Groups of 12 were sprayed outdoors for a minute. During the spraying, the subjects exercised in various ways; some were kept in wet clothing for an hour to investigate the effects of protracted contact with the solution. The subjects reported that stinging of the eyes was followed by stinging of the skin, first of the face, then the neck, the back, and the lower body. Irritation was mild and disappeared in about 10 min. Before and after the drenches, blood pressure was measured. The control drenches with water alone caused a transitory rise in blood pressure, but the effect of the 0.005% CS spray was a mean rise of 31 ± 2.7 mm Hg systolic and 19 ± 2 mm Hg diastolic. It took an average of about 6.3 min for the pressure to decrease to nearly normal--close to the time it took for skin sensation to end. When the CS drench was followed by exercise, the systolic pressure rose as before, but the diastolic pressure decreased. The authors concluded that, although a cold-water drench alone provoked a brief rise in pressure, it was only partly responsible for the rise seen during the CS drench. A direct hypertensive effect of CS was ruled out, inasmuch as the rise in blood pressure came too rapidly to be accounted for by the absorption of a substantial amount of the agent. The rise must therefore have been caused by the intense irritant effect of CS.

Tolerance

In an atmosphere containing CS at low concentrations, men can remain without discomfort for long periods. Punte *et at.*[32] reported that men can tolerate CS at 1.5 mg/m^3 for at least 90 min; men can tolerate 6.6 mg/m^3 if the concentration is built up over 30 min. In an atmosphere of 6 mg/m^3, built up in 10 min, three men were forced to leave the chamber in 18, 20, and 29 min; the fourth stayed in for 40 min, at which time the test was ended. The Himsworth committee[20] reported a similar experiment in which 35 men were exposed to CS gradually built up to 2.30 mg/m^3 over an hour; all but two remained. Such tolerance is lost quickly. An adjusted person who leaves the chamber for a short time is strongly affected on returning.

The ability to remain in a contaminated atmosphere depends on will power and motivation, as well as the dosage tested. McNamara *et al.*[28] measured ICt_{50}s for men in a variety of conditions. Subjects were instructed to remain in a wind tunnel facing a CS spray (5% in acetone) until they could no longer tolerate it, at which time

TABLE 4-16

Ability of Subjects to Tolerate Exposures to 1-μm and 60-μm Particles of CS Aerosols[a]

Exposure of:	Percentage of Subjects Tolerating CS for 1 min: Small Particles	Percentage of Subjects Tolerating CS for 1 min: Large Particles
Eyes	40	100
Respiratory system	0	67

---

[a] Data from Owens and Punte.[30]

TABLE 4-17

Mean Recovery Time of Subjects after Exposure to 1-μm and 60-μm Particles of CS Aerosols[a]

Exposure of:	Mean Recovery Time, s: Small Particles	Mean Recovery Time, s: Large Particles
Eyes	91	280
Respiratory system	51	9

---

[a] Data from Owens and Punte.[30]

they emerged. Incapacitating concentrations recorded for two experiments were as follows:

	ICt_{50}, mg/m^3
a. 78 men, merely asked to remain in the wind tunnel as long as possible	3.0, 4.7
b. 35 men, no motivation	0.1, 0.07
c. 30 men, same as a	0.3, 0.2

d.	21 men, same as b	0.7, 0.6
e.	130 men, motivated by special psychologic techniques	12.4, 6.9
f.	30 men, same as a	1.4, 0.5

Money can be effective motivation. At the British Chemical Defense Establishment at Porton Down, Wilts., volunteers were told that a 5-pound note could be found in a hut.[10] A CS cartridge was exploded in the hut and the men were allowed to enter to look for the money. The men could tolerate concentrations of agent probably in the range of several hundred milligrams per cubic meter. With sufficient motivation, then, men can endure exposure to CS at high concentrations. No harmful after-effects occurred in the tests described or in rioters who may heve been exposed long enough to accumulate rather high doses of CS.

Beswick *et al*.[10] exposed 35 men in small groups to steady and increasing concentrations of CS to study tolerance. In two trials, there were eight men in the chamber. Four wore masks until the last 5 min of the hour and then unmasked to demonstrate the difference between their reactions and those of the four who had become accustomed to the agent. In 10 trials, only two men were forced to leave the chamber because of nausea. After about 5 min, men reported that symptoms were more bearable; some were able to play cards. When the CS concentration was increased, most reported a temporary increase in symptoms. The highest exposures (Ct's between 60 and 90 $mg \cdot min/m^3$) were higher than would be experienced by rioters.

Sensitization

Guinea pig tests indicated that CS had a potential for producing human skin irritation and sensitization.[38,39]

Fisher,[14] discussing tear gases and their effect on human skin, remarked that CS is a sensitizer and a primary irritant capable of causing first- and second-degree burns and even ulcers if not washed off the skin. In experimentally sensitized subjects, CS elicited a skin response in one of nine when tested at 0.1%, but none reacted at 0.01%.[27] Concentration is a factor in the elicitation of a skin response to a sensitizer, as well as an irritant.

Thus, it is not surprising that munitions plant workers in both England and the United States developed rashes, dermatitis, and blisters from contact with CS powder.[11,41] Protection of these workmen was achieved by the use of air-supplied suits and rigorous attention to bathing and changing of clothing after work. Bowers *et* al.[11] noted that no one seemed immune to the effects of CS, although some appeared more sensitive than others. Bowers et al. reported case histories of 11 men who developed contact dermatitis after exposures to CS from 2 d to 2 mo. There appeared to be a wide range of sensitization potential, greater in white than in black workers. Five men developed hypersensitivity after repeated exposures. Others suffered various degrees of dermatitis, pruritus, and vesiculation in proportion to exposure. High heat and relative humidity intensified these effects.

Shmunes and Taylor[41] reported contact dermatitis in a plant manufacturing CS. Of 28 workmen, 25 (89%) had dermatitis of some degree in one or several episodes. Nearly all began to suffer from dermatitis 2 wk to 6 mo after the original exposure to CS. Although protective clothing was worn, carelessness in sealing neck and wrist junctions and in changing clothing at the end of the workday seemed to cause the dermatitis. Most of the lesions were on the neck and wrists. Patch tests to detect allergic sensitiztion to CS showed that only two of the 25 subjects reacted.

Accidental Exposures

Park and Giammona[31] reported the effects of a 2- to 3-h exposure to CS on a 4-mo-old child. The infant was in a house into which police fired several canisters of CS. No estimate of concentration was available. On admission to the hospital, the child was suffering from severe respiratory distress and first-degree burns on the cheeks. Despite a week of treatment, pneumonia developed. The child was released after 28 d of hospital care.

USE IN NORTHERN IRELAND

Himsworth Report, Part I

On August 13 and 14, 1969, extensive rioting broke out in Londonderry, Northern Ireland. CS was used in large quantities (a total of some 14 kg). An official inquiry into the medical hazards of such riot-control measures was instituted. The findings of the committee were published in September 1969[19] and September 1971.[20] Part I of the "Himsworth report" is devoted to an account of a 3-d investigation (September 1-3, 1969) in the area of the riots. A three-member committee, later expanded to eight members, interviewed the local public-health authorities, hospital officials, physicians, and many inhabitants of the district. Given the chaotic situation, no exact information on exposure conditions or immediate after-effects could be expected. A decision was made to gather information on two groups of exposed people: those who had previously appeared healthy and those who had some illness that had been exacerbated by the exposure to CS. Part I concluded:

> The conclusions that we have been able to reach in respect of the effects of CS in the circumstances, and under the conditions existing in Londonderry during the incidents of the 13th and 14th August 1969, necessarily vary in their firmness. We feel reasonably confident in the conclusions that we have drawn in regard to effects of exposure to CS in previously healthy persons. We feel that our conclusions in regard to the effects on persons who had certain previously established illness are more open to question, although we believe that they could be rated as strong possibilities. Our conclusions in respect of the future course of illnesses that had been present before exposure are necessarily more tentative. With these provisos we summarise our main conclusions below.
>
> We have found no evidence even among those most heavily exposed to CS of incapacitation as a result of exposure such as to prevent their moving away to a clearer atmosphere where the acute symptoms rapidly abated.
>
> We have similarly found no evidence in previously healthy persons that, following exposure, any illness developed in the following three weeks that could clearly be attributed to the effects of CS. The most common complaint was of mild diarrhea but there are certain features about the incidence of this that make us hesitate to ascribe it to the effects of CS.
>
> In respect of persons who were in ill health previous to exposure, we have paid particular attention to cases of asthma, chronic bronchitis and emphysema. We believe that it must be accepted in principle that exposure to CS may precipitate an acute asthmatic attack, but we found no evidence that such attacks differed in kind or degrees from those attributable to natural causes and, in the cases that we either saw or heard of, we were unable to exclude the possibility that the attacks in mid-August were due to such causes. We must accept the possibility that exposure to CS of a patient with chronic bronchitis and emphysema may result in an acute bronchitis being superimposed on the chronic condition. Again this does not appear to differ from the situation when such an acute exa-

> cerbation occurs from natural causes. In reaching an assessment of the possible effects of the present acute episodes of illness associated with exposure to CS upon the future course of the previously established chronic disease, we feel, therefore, that it would be reasonable to suggest that these are not likely to differ from the effects of comparable acute episodes produced by natural causes.
>
> Finally, we would express a general opinion to which we have come in respect of chemical agents that might be used for civil purposes. In our opinion, the point of view from which the effects of any such agent should be studied should be more akin to that from which we regard the effects of a new drug than to that from which we might regard a weapon. We are aware that over recent years opinion has tended increasingly towards this view and that investigations have been made with such safeguards in mind. But we feel that this view should now graduate from the status of a tacit understanding to that of an explicit requirement. To that end, the effects of any such agent should be appropriately investigated, not only in respect of the healthy persons against whom it may be directed, but also in relation to the possible effects on the young, the elderly and those with impaired health, who may inadvertently be exposed to the agent in question. It is primarily for this reason that we have recommended and it has already been agreed by the Home Secretary that our membership should be expanded and the evidence in regard to CS assessed in the widest possible way.

Himsworth Report, Part II

Part II of the Himsworth report dealt with the toxicity of CS in healthy subjects and with regard to pre-existing disease. Because exposures to CS in riot-control situations, as in Londonderry, are brief but often intense, special attention was given to such exposures.

The lowest concentration detectable by man is about 0.004 mg/m^3. The concentration that would disperse a crowd of rioters was estimated at about 4.0 mg/m^3, and the amount to deter trained troops, at 10 mg/m^3. Above the latter concentration, no increase in the severity of symptoms was detectable.

Animals are less sensitive than humans. When exposed to CS, animals pass through an excitatory phase, after which they usually lie passively. Animals exposed to CS show severe congestion of the blood vessels in the lungs. "With concentrations approaching those that might occur in riots, neither illness nor lung damage is demonstrable."

The report on the effects of CS on healthy people considered ordinarily healthy persons (such as would most likely join in riots) and special groups. No indication had been found during the visit to Londonderry that rioters had suffered marked after-effects, on the basis of interviews with area residents and medical authorities. To obtain reliable information on CS effects, a test was arranged in which 34 human volunteers were exposed to CS for an hour, accumulating a total dose of "90 mg·min/m^3," which is interpreted as 1.5 mg/m^3 for 60 min. Blood samples were taken before and immediately after the exposure and 24 h later, so that hematologic and biochemical postexposure values could be compared with the normal.

Some changes were registered in the hematologic and biochemical values, but they were not regarded as deleterious to the subjects.

Among the special categories of healthy persons were the young and the aged. No cases of illness attributable to CS were found among infants, even among those who were mildly exposed by accident. Similarly, old age as such seemed not to be predisposing.

Effects on pregnancy were sought by examining statistics for abortions, stillbirths, and congenital abnormalities. These statistics gave no indication of any increase in abortions, stillbirths, or congenital abnormalities.

The Himsworth committee visited Londonderry in July 1970 and investigated the health of those known to have been suffering from various illnesses before the riots, who might have been affected by exposure to CS. A primary concern was for adverse reactions in people with respiratory diseases, such as chronic bronchitis and asthma. Although exposure to CS had exacerbated effects in patients with chronic bronchitis, a followup visit showed that they had returned to their preriot health status. Much the same results were found with asthma patients. No increase in the frequency of attacks had been noticed.

The committee examined other diseases that might have been increased by exposure to CS. Most remarkable were the data on new tuberculosis cases for the year after the riots: the rate had increased for all Londonderry districts except the Bogside, where the riots had occurred.

Although exposure to CS causes a temporary rise in blood pressure,[17] no increase in cases of stroke or heart conditions was reported. The committee speculated that this might have been because exposure to CS does not increase airway resistance[32]--increased airway resistance increases the strain on the heart--and because people with known heart problems were unlikely to take part in rioting.

For a short time after the riots, men who had been suffering from epilepsy and were being controlled by treatment began to have attacks. The committee speculated that, in the excitement of the riots, epileptics had neglected to maintain their medication. No unusual occurrences of psychiatric or other mental disorders seem to have been recorded (see the following section, on sequelae of the Belfast riots).

The committee devoted much effort to the likelihood of deaths in riots as a result of high local concentrations of CS either outdoors during combat or indoors by accidental penetration of a house window. It concluded that in the open air it would be impossible for a healthy human to receive a lethal dose, because even light winds disperse CS and the burning time is short for cartridges (10-15 s) and grenades (25 s). The exposure concentration for a man at whose feet a missile exploded was estimated as 100-300 mg/m^3. Indoors, in a 20-m^3 room, the explosion of a standard cartridge (12.5 g of CS) produced a concentration of 500 mg/m^3 when the room was closed. Assuming a broken window or an open door, the concentration would diminish rapidly, so that even someone unable to leave the room would not be exposed to a dangerous amount of CS.

Jones,[23] in a critique of the Himsworth report, recommended abandonment of CS as a riot-control agent, because lethal amounts could be inhaled under "the most exceptional circumstances."

Mental Effects of Riots

Fraser[15] studied institutional admission rates and outpatient referral rates for Belfast during the riots of 1969 and compared the rates for psychoses and neuroses with those of the previous year. The city was divided into three areas: Area 1 was the district in which the rioting had occurred; Area 2 was the adjacent territory, in which little violence occurred, but which exhibited signs of tension, such as boarded-up windows and barricades; and Area 3 had been free of disturbance and was small relative to the rest of Belfast.

The data are remarkable. The rates for Area 1 remained almost constant, but the rates for male psychotics and for male and female neurotics increased in Area 2. Statistically, the changes were marginal, but Fraser believed that late referrals might have increased the differences. He suggested that the anticipation of violence in Area 2 provided stress that caused increases. He compared this with similar data compiled for wartime England, in which admission rates rose in the provincial areas, but not in London under the bombing. Fraser examined the numbers of prescriptions for tranquilizers issued in Belfast. Increases were noted in Area 1, but not in the other areas.

Lyons[25] studied the psychiatric sequelae of the Belfast riots, focusing on the period August 15-September 30, 1969. He concentrated on 257 patients from three general medical practices in the riot area of West Belfast and on patients referred to psychiatrists from these general practitioners. He concluded that during civil disturbances:

- There is no increase in acute psychiatric illness.

- Very young and very old people are less likely to develop mental symptoms than young to middle-aged adults.

- Unemployed people are at more risk than working people.

- Women predominate (about 75%) among the patients.

- People living alone are at less risk than others.

Neither Fraser nor Lyons correlated his study with exposure to CS, but the widespread use of CS and the public clamor resulting from this new aspect of riot control must have been an important factor in the stress that operated on the inhabitants of Belfast, both within and outside the combat zone. It seems worth while, therefore, to include the record of psychiatric effects, even without a direct connection to the use of CS.

LONG-TERM FOLLOWUP

Marrs *et al.*[26] exposed 300 mice, 200 rats, and 200 guinea pigs, all males, to CS at high, medium, and low concentrations in aerosol form 1 h/d, 5 d/wk for 120 d. The aerosols were generated from pure CS and had a mass median diameter of 3-4 μm. Mice were exposed 55 times (11 wk), rats and guinea pigs, 120 times (24 wk). The high-dose animals began to die after a few exposures, so that series was terminated. All survivors in other groups were killed at the end of a year and necropsied.

Because of deaths at the high concentration, the middle-dose animals received higher total doses, and there was no significant mortality. Low- and medium-dose animals showed no greater mortality than controls. No indications of a dose-response relation between tumors in any particular site and the total dose of CS were found. Marrs et al. concluded that CS below 30 mg/m^3 is not harmful to mice, rats, and guinea pigs. This is more than 8 times the concentration (IC_{50}) intolerable to 50% of an exposed human population in 1 min.

Little is known about possible long-term effects of CS inhalation. This is due in part to the fact that short-term experiments with experimental animals, carried out for from several days to a month and using much higher (in some cases, nearly lethal) concentrations of CS, showed that ocular, respiratory, and cutaneous alterations were mild and readily reversible, whereas necropsy findings failed to reveal any evidence of systemic alterations. Retrospective studies performed by the Himsworth committee at the request of the British Parliament after the extensive use of CS in Northern Ireland showed that no adverse effects of CS use were observed, with respect to eye burns, residual respiratory tract injury, increased death rate in the elderly, exacerbations of mental illness, increased incidence of strokes or heart attacks, or incidence of tuberculosis. At exposure concentrations reported by the Himsworth committee (about 90 $mg\cdot min/m^3$), no persistent or notably adverse health effects were observed.

Experimental studies carried out in animals and human volunteers exposed to CS aerosols revealed no lasting changes in hematologic or biochemical measures. CS exacerbates symptoms of pre-existing chronic bronchitis and bronchial asthma, but these rapidly return to a pre-exposure state after removal of CS.

Two instances of accidental massive exposure have been recorded, one of a healthy 43-yr-old man, and another of a 4-mo-old child. Both experienced severe respiratory symptoms (pulmonary edema in the first, and later pneumonia in the second), but they reportedly recovered. No long-term followup data are available.

EFFECTS ON HUMAN SUBJECTS AT EDGEWOOD

From 1958 to 1973, at least 1,366 human subjects underwent experimental exposures to CS at Edgewood. For 1,073 subjects, there was some type of aerosol CS exposure, 180 subjects had skin applications, 82 subjects had both skin applications and aerosol exposures, and 31 underwent CS applications to their eyes.

Earlier subjects underwent up to 10 CS exposures in the wind tunnel on different days, but later experiments involved a maximum of three exposures. Most of the CS exposures involved tests of equipment or of subjects' abilities to perform military tasks during exposure. Other protocols combined effects of stress, motivation, and other substances and used tests of effectiveness of samples of CS. There was a wide range of exposure Ct's--from 0.03 to 345 mg·min/m^3. The highest Ct's were used in equipment studies. (Protocol and Ct information is not available on all subjects.)

Complete experiment records are available on 105 CS subjects (Table 4-18); a summary is available on an additional 86 subjects who participated in CS skin-sensitization experiments in 1972. The records represent a cross-section of many of the CS protocols. The amount of information in each record varies with the protocol. Ct's, where mentioned in the 105 records, ranged from 7 to 345 mg·min/m^3. Exposure times, where mentioned in the 105 records, ranged from 18 s to 10 min. In motivation experiments, subjects attempted to remain in CS for up to 200 s, but many left before test completion. Dosages in skin tests were 0.01 or 0.025 ml of 1% CS applied to bare or clothed arms.

Among the 105 subjects, signs and symptoms due to CS exposure were most marked in the eyes and respiratory tract. All effects were temporary. Frequent symptoms, all temporary, were lacrimation, eye irritation, upper respiratory passage irritation, chest constriction, and dyspnea. The main objective findings mentioned were conjunctivitis and rhinorrhea. Pulmonary examinations were generally not mentioned. Other symptoms included headache, nausea, and diarrhea. The eight skin applications on which records are available reportedly caused no effects.

Postexposure (generally 1 wk) laboratory analyses (complete blood count, complete urinanalysis, blood urea nitrogen, alkaline phosphatase, and serum glutamic oxalacetic transaminase) are available on 50 of the 105 subjects. An additional 22 earlier subjects' records contain results from a less complete series of postexposure laboratory analyses. Pre-exposure laboratory analyses were used for comparison on subjects with abnormal postexposure laboratory results. Eleven subjects had laboratory abnormalities that were not seen before exposure to CS. There were seven subjects with urinary sediment containing 2-10 white cells per high-power field. There are no reports of a lower urinary tract source of white cells in these seven subjects. Results of their other postexposure renal-function tests were normal. Three subjects had abnormally high serum glutamic-oxalacetic transaminase (112, 38, and 31 IU). Leukocytosis accompanied the marked increase in transaminase in one subject. One subject had leukopenia (2,800 cells with a normal differential count). None of the subjects with abnormal postexposure laboratory results had earlier experimental exposures at Edgewood, although some participated in later experiments.

In 1969, 31 subjects had ocular instillations of either 0.1% or 0.25% CS in water with 0.5% polysorbate 20 or 0.05- 1.0% CS in trioctyl phosphate in their right eyes. The subjects experienced intense ocular irritation and lacrimation. Acute conjunctival injection lasted 1 h. No fluorescein staining of the cornea was seen under ultraviolet illumination. In one subject, corneal staining, visualized with the slit lamp, resolved in 24 h.[34,35]

In 1972, skin applications of 0.01% and 0.1% CS were used to determine the effects of skin pigment on susceptibility to sensitization, sensitizing and irritating concentrations of CS, and cross-sensitization with CN. Eighty-six tests were summarized, and results are available on 45 cases. Seven of 15 subjects were sensitized in skin-pigment experiments; subjects with lighter skin seemed more susceptible to sensitization. Twenty previously CS-sensitive subjects showed no cross-reactivity to CN, although 18 developed primary irritation dermatitis when exposed to 0.2% CN. Four of 20 subjects developed primary irritation dermatitis when exposed to 0.1% CS, but not to 0.01% CS.

There are no data to suggest that the low dose CS-exposure of 105 subjects at Edgewood would give rise to long-term health effects in the primary target organs, the eyes and respiratory tract. CS is a known skin sensitizer, causing allergic contact dermatitis after repeated exposures in a high percentage of subjects. Many Edgewood CS exposed subjects were probably sensitized to CS; in fact, many known sensitized persons were chosen for some protocols. Sensitizing effects of CS on other organs are not known, but the risk of allergic pneumonitis on exposure of a sensitized person to CS is a possibility.

Hepatic dysfunction and urinary abnormalities were seen in some subjects after CS exposure at Edgewood. Little is known of the effects of CS on the kidneys and liver. The small proportion of subjects who had abnormal urinalysis (7 of 50; 14%) and high transaminase (3 of 50; 6%) indicates idiosyncratic reactions, if the abnormalities were indeed due to CS exposure. The most likely course of idiosyncratic drug-induced, nonfulminant hepatitis is complete recovery after removal of the agent. Recurrence of hepatic reactions would be expected on re-exposure to CS if the original transaminase increases were due to CS.

In summary, the available data on Edgewood CS-exposed subjects lead to speculation about several possible health effects. Repeat exposures to CS may cause allergic contact dermatitis in many of the Edgewood subjects. One could speculate that repeat exposures to CS may also induce idiosyncratic hepatitis or allergic pneumonitis in some persons, although no evidence of this exists with the Edgewood subjects.

TABLE 4-18

Test Results Involving 105 CS Exposure Subjects on Whom Complete Records are Available

Mo/Yr of Exposure	No. Subjects/ No. Times Exposed	Mode of Exposure or Protocol	Ct, mg.min/m^3	Time	Conc.	Symptoms and Signs	Laboratory Analysis[a] No. Subjects and Type Test	Abnormal Results
4/58	1/4	--	--	18-120 s	--	Blurred vision, lacrimation, conjunctivitis, choking, cough, dyspnea	0	--
11/58	1/2	--	--	--	--	"Usual"	1(BSP)	0
2/59	6/10	Wind tunnel	--	--	0.5-6 ppm	Throat and chest irritation, choking, dyspnea, conjunctivitis, lacrimation, no tolerance	6(NPN), sodium potassium, alk. phos., thymol turbidity, urine (protein)	0
3,4/59	32/1 or 2	Wind tunnel (masked) and aerosol grenade	20-111	--	--	Eye irritation, conjunctivitis, skin irritation, chest constriction, cough, rhinorrhea, headache, nausea (2), diarrhea (1)	15(BSP, NPN)	0
7,12/67	6/1	Wind tunnel	21-50	--	--	Eye irritation, lacrimation, skin irritation, nose and throat irritation, rhinorrhea, chest constriction, dyspnea	6	1 urinary WBCs
5/68	16/1	Motivation	25-52, up to 10 min	--	--	"Typical" ocular, nasal, respiratory effects	15	2 urinary WBCs; 1 leukopenia
	7/1	Skin application	--	--	0.01 or 0.025 ml of 1% CS	"No effects"	4	0
7/68	4/1	Motivation	7-59, up to 200 s	--	--	"Usual" ocular, nasal, respiratory effects	3	1 SGOT and leukocytosis
10,11/68	6/1	Simple testing	20-100	--	--	--	4	2 urinary WBCs
12/68-2/69	5/1	Motivation	23-24	--	--	--	5	1 SGOT
4/69	1/2	Aerosol	20 and 35	--	--	--	1	0
11/70	6/1	+ diazepam	--	--	--	--	2	0
	10/1	+ atropine	--	--	--	Dry mouth, blurry vision, eye irritation, headache, nausea, nervousness, malaise, dizziness,	10	1 SGOT 1 hematuria/ pyuria 1 glycosuria
12/70	4/1	Equipment	311 and 345	--	--	"No leaks"	0	0

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[a] Unless specific tests are mentioned, laboratory analysis included CBC, complete UA, BUN, alkaline phosphatase, and SGPT. Abnormal results are recorded only if pre-exposure laboratory analysis did not reveal abnormality.

SUMMARY

CS has been widely adopted, especially in the United Kingdom, as a replacement for CN for use as a tear gas or riot-control agent. This has come about because careful investigation has shown that CS is less toxic than CN, acts faster, and has after-effects of shorter duration and less severity. Both humans and animals have been subjected to extensive investigation. There is a wide safety margin between the lowest concentration that is effective in disabling rioters and the concentration that could cause life-threatening harm to the exposed. There is virtually no evidence that CS poses a mutagenic or carcinogenic hazard. There are no reports of death from CS exposure, as there have been for CN. Thousands of men have been exposed to CS in the course of military training, with few after-effects. The Himsworth committee was unable to locate anyone exposed to the effects of CS during the Londonderry riots who suffered important sequelae, except for temporary exacerbation of chronic bronchitis or asthma. CS has one lasting effect: In addition to being a primary skin irritant capable of causing first- and second-degree burns, it is a skin sensitizer; once exposed to CS, many develop allergic reactions on later contact. This is a problem particularly for industrial workers who are in contact with CS daily.

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CHLOROACETOPHENONE

CHARACTERISTICS

Chloroacetophenone (Mace, 1-chloroacetophenone, o-chloroacetophenone, CAP, phenyl chloromethyl ketone), a lacrimator, is a white volatile crystalline solid with an odor like that of apple blossoms. It is commonly referred to as CN. It melts at 54°C, boils at 245°C, is slightly soluble in water, and is soluble in ethanol, benzene, acetone, and benzyl chloride.[30] Its vapors are extremely irritating to the eyes and skin and may cause permanent injury to eyes.

CN causes alkylation of sulfhydryl-containing enzymes, acts as an enzyme inhibitor, and has a denaturing effect on tissue proteins.[10,20,22] It is an alkylating agent of the S_N2 type (substitution nucleophilic, second order).

CN is generally considered to be moderately toxic, and more toxic than CS. It is used in much the same way as CS--sprayed in a liquid carrier, as a micropulverized powder, or in pyrotechnical grenades. In recent years, pen guns and small pistols carrying CN cartridges have become popular as personal defense weapons. Injuries have resulted from their careless use. As of June 1, 1975, pen guns were classified as firearms and placed under the controls and restrictions of the Gun Control Act of 1968 by the Bureau of Alcohol, Tobacco, and Firearms, Department of the Treasury.

TOXICOLOGY IN IN VITRO AND ANIMAL STUDIES

Lee and Webber[19] treated HeLa cells with CN and studied effects on cell morphology. CN at concentrations of 1.5×10^{-5}, 3.8×10^{-5}, and 7.6×10^{-4} mol/L was added to HeLa cell cultures for 1.75 and 3.5 h and then washed out. The cultures were incubated for 7 d. Growth was measured in terms of total purines and pyrimidines, and the concentration required to cause inhibition of growth by 50% was estimated as 10^{-5} mol/L. Variations from the normal cell form, as well as early degenerative changes, were found. Lee and Webber attributed these effects to inhibition of cellular enzymes through interactions with sulfhydryl groups.

Lakshmi[17,18] studied the effects of CN on chick embryos. Embryos at the primitive-streak stage (18 h) and at the head-process stage (22 h) were treated with CN at 5×10^{-4} M for 15 min and examined after further incubation for 21 h. Primitive-streak embryos had malformations of the brain (81.4%) and of the neural tube (48.7%). Those treated at the head-process stage were unaffected by the CN treatment. Lakshmi attributed the injuries to inhibition of sulfhydryl-containing proteins during morphogenesis. Apparently, no injury resulted from CN treatment in the head-process stage, because the invagination of the chorda-mesoderm and induction of the nervous system had taken place.

Lakshmi treated primitive-streak stage embryos with CN to study the effect on Hensen's node (related to the organization of the embryo). The embryos were incubated for 3 h after treatment; the nodes were then excised. washed, and grafted to host chick embryos at the same stage. The results showed an inverse relation between the capacity for induction of Hensen's node and exposure to CN.

McNamara et al.[25] summarized the LCt_{50}s for three animal species exposed to CN as a dry dust and in sprayed solutions (Table 4-19). For a comparison of single and repeated exposures, 20 guinea pigs and eight monkeys were exposed to CN at a Ct of 2,300-4,000 mg·min/m^3 on each of 10 consecutive days. The cumulative Ct was 31,445 mg·min/m^3. In a single exposure, this Ct would have killed most of the animals. Only five guinea pigs died. Eight dogs were exposed to CN at a Ct of 3,000-7,000 mg·min/m^3 on each of 10 consecutive days. The cumulative Ct was 60,000 mg·min/m^3, enough to kill all the dogs if it had been a single exposure. Only one died. In another test, 20 guinea pigs, eight dogs, and eight monkeys were given a cumulative Ct of 88,000 mg·min/m^3 in 10 daily exposures; few animals died. These tests showed that CN is detoxified rapidly and has little cumulative toxicity.

TABLE 4-19

Lethal Effects of CN in Three Animal Species

Species	No. Animals	LCt_{50} mg·min/m^3
Rats	190	8,878
Guinea pigs	106	7,984
Dogs	62	7,033
Total	358	6,189

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[a] Data from McNamara et al.[25]

LD_{50} data for several species of animals and several irritants are in Table 4-1(

Striker et al.[36] exposed 30 Macaca mulatta monkeys to CN, 10 at each of three exposure conditions, as shown in Table 4-20. Two monkeys at each Ct were killed and autopsied at 12 h, 24 h, 72 h, 7 d and 30 d. At the low dose, pathologic changes were greatest from 24 to 72 h; animals autopsied at 30 d had no lesions. The middle dose, however, caused increased pulmonary edema; the high dose resulted in hemorrhages. Permanent damage was caused at the two higher doses; three spontaneous deaths occurred 24 h after exposure at the high dose. These doses were far higher than would be encountered in an open-air situation, such as a riot; but they might be approached in a closed space if several grenades were exploded.

Ballantyne and Swanston[4] examined the acute toxicity of CN administered by several routes in mice, rats, guinea pigs, and rabbits. Tables 4-21 and 4-22 present the results for oral, intravenous, intraperitoneal, and inhalation exposures. The animals that died from oral administration usually did so 2-18 h after dosing. They showed congestion of the lungs, stomach, and intestine (with erosion of the mucosa) and congestion and hemorrhages of the thymus. Kidney and liver necrosis was seen in many animals. Intravenous administration resulted in congestion of liver, kidneys, lungs, spleen, thymus, and eyes. Surviving animals recovered in about 3 wk and showed no abnormalities when autopsied. Intraperitoneal CN was more toxic in guinea pigs, but about as toxic as intravenous CN in rats. Congestion in the viscera was much the same as that caused by other routes of administration. Animals that died after inhalation of CN had congestion of the alveolar capillaries and intrapulmonary veins, alveolar hemorrhages, and excessive secretion in the bronchi and bronchioles. There were areas of patchy acute inflammatory cell infiltration of the trachea, bronchi, and bronchioles.

In skin-irritation tests, Ballantyne and Swanston found that guinea pigs were more sensitive than rats with regard to erythema, but less so for edema. Rabbits were more affected in both categories.

Ballantyne et al.[3] examined the ocular effects of CN on rabbits. Results of tests with CN at 1-10% in polyethylene glycol (PEG) showed that severity and duration of effects were concentration-related. Lacrimation, chemosis, and iritis were more severe and persistent when the vehicle for CN was corn oil and PEG than when it was trichloroethane (TCE) or trioctylphosphate (TOF). Blepharitis was most severe when CN was dissolved in corn oil. CN dissolved in corn oil or PEG caused a greater degree of keratitis than that dissolved in TCE or TOF.

Gaskins et al.[8] investigated the acute toxicity of CN given by stomach tube in various solvents in male and female rats. Moderate to severe gastroenteritis was observed, often followed by death

TABLE 4-20

Summary of Lesions Found in Monkeys Exposed to CN[a]

	No. Monkeys Showing Lesions														
	Ct 3,025 mg·min/m^3 (1,219 mg/m^3, 2.5 min)					Ct 9,251 mg·min/m^3 (1,682 mg/m^3, 5.5 min)					Ct 21,699 mg·min/m^3 (2,411 mg/m^3, 9.0 min)				
Lesion	12 h	24 h	72 h	1 wk	30 d	12 h	24 h	72 h	1 wk	30 d	12 h	24 h[b]	72 h	1 wk	30 d
Pulmonary edema	—	—	1/2	—	—	1/2	2/2	—	2/2	—	2/2	3/3	2/2	1/2	—
Pulmonary congestion	—	—	1/2	1/2	—	1/2	2/2	2/2	1/2	—	—	3/3	—	—	—
Pulmonary hemorrhage	—	—	1/2	—	—	—	—	—	—	—	—	3/3	—	—	—
Cardiac hemorrhage	—	—	—	—	—	—	—	—	—	—	1/2	1/3	—	—	—
Bronchorrhea	2/2	1/2	1/2	—	—	1/2	2/2	—	1/2	—	—	—	—	—	—
Bronchitis	—	2/2	1/2	—	—	2/2	2/2	—	1/2	—	2/2	—	1/2	—	1/1
Pneumonia	—	—	—	—	—	—	2/2	—	—	2/2	—	—	2/2	1/2	1/1
Emphysema	2/2	1/2	2/2	2/2	—	1/2	2/2	2/2	1/2	2/2	1/2	3/3	2/2	2/2	—
Atelectasis	—	—	—	2/2	—	1/2	—	1/2	1/2	2/2	—	—	—	1/2	—
Froth in trachea and bronchi	2/2	1/2	1/2	—	—	1/2	2/2	2/2	1/2	—	1/2	1/3	1/2	1/2	—
Edema of larynx	—	—	—	—	—	—	—	—	—	—	1/2	1/3	—	—	—
Aspiration	2/2	—	—	—	—	—	—	—	1/2	—	1/2	—	—	—	1/1
Hemorrhagic gastroenteritis	—	—	—	—	—	—	—	—	—	—	—	1/3	—	—	—
Corneal ulcer	—	—	—	—	—	—	—	—	—	—	—	—	2/2	—	—
Tracheitis	—	—	1/2	—	—	—	—	—	1/2	—	2/2	—	2/2	—	—
Interstitial inflammatory cell infiltration	—	—	—	—	—	—	—	—	2/2	—	—	—	—	—	—
No lesions	—	—	—	—	2/2	—	—	—	—	—	—	—	—	—	—

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[a] Data from Striker *et al.*[36]

[b] Three monkeys died spontaneously, leaving only one in this dosage group to be killed at 30 d.

TABLE 4-21

LD_{50} Values of CN Administered Orally, Intraperitoneally, and Intravenously in Rats, Rabbits, Mice, and Guinea Pigs[a]

Route	Species	Dosage, mg/kg	LD_{50} (5% confidence limits), mg/kg
Intravenous	Rabbit (male)	25-100	31 (25-35)
	Rabbit (female)	24-48	30 (16-39)
	Mouse (male)	40-113	81 (66-109)
	Rat (female)	25-63	41 (36-49)
Intraperitoneal	Rat (male)	30-50	36 (26-47)
	Guinea pig (female)	14-40	17 (5-22)
Oral	Rat (male)	100-400	127 (113-144)
	Rabbit (female)	78-200	118 (69-143)

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[a] Data from Ballantyne and Swanston.[4]

TABLE 4-22

Mortality in Rabbits, Mice, and Guinea Pigs after Inhalation of CN[a]

Species	Duration of Exposure, min	Average Concentration, mg/m^3	Ct, $mg \cdot min/m^3$	No. Exposed	14-Day Deaths
Rabbit (male)	15	417	6,255	20	11
	15	593	8,895	20	6
	20	559	11,180	20	10
	25	651	16,275	20	16
	30	742	22,260	15	14
	37	749	27,713	20	19
Rabbit (female)	20	465	9,300	8	3
	15	638	9,570	10	0
	15	665	9,975	10	2
	15	720	10,800	10	4
	30	423	12,690	10	7
	30	652	19,560	5	5
	30	742	22,260	5	5
	60	551	33,060	10	10
Mouse (female)	15	600	9,000	50	7
	15	640	9,600	50	6
	30	592	17,760	52	11
	30	652	19,560	22	5
	37	719	26,603	51	20
Guinea pig (female)	15	243	3,645	20	0
	20	465	9,300	10	0
	30	490	14,700	20	16
	30	667	20,010	20	18
	30	764	22,920	20	20
	60	627	37,620	20	20

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[a] Data from Ballantyne and Swanston.[4]

(Table 4-23). They tested rabbits for eye and skin irritation. A 1% w/v solution of CN in TCE caused a reddening of the eyes for 48 h. Washing of the eyes 10 min or less after treatment almost eliminated chemosis. Because the aim of these experiments was to estimate the harmful effects of commercial formulations (Mace, etc.), 25 formulations were tested. Only one, which contained 4.3% w/v of CN, produced permanent eye injury in rabbits. Others, containing 0.04-2%, produced no permanent eye or skin injury. Testing for skin irritancy was performed by applying 0.5 ml of various commercial CN-containing preparations on either intact or abraded rabbit skin, with patches covering the areas for 24 h. This usually produced a necrotic eschar 5-6 d after treatment. Washing of the skin did not prevent further injury, even if done 10 min after application. Skin recovery took 3-5 wk.

McNamara et al.[25] tested corn oil dilutions of CN in topical applications of 0.05 ml to rabbit eyes and skin. The results were as follows:

Eyes: 0.5 mg--no effect
1.0 mg--transitory conjunctivitis
5.0 mg--corneal opacity
Skin: 5.0 mg--erythema and necrosis

Rothberg[33] studied sensitization to CN in guinea pigs. Using the Landsteiner technique, he applied acetone solutions of CN both topically and by intradermal injection. Twelve induction doses were given to eight animals by each route. After a 2-wk rest period, challenge was made topically at a fresh skin area. There were seven positive reactions in each group of eight animals. Sensitization to CN also occurs in humans.[24,29]

Gwynn and Salaman [11] tested a number of compounds, including CN, as possible promoters of skin carcinogenesis when 9,10-dimethyl-1,2-benzanthracene (DMBA) was used as an initiator. Treatment consisted of 0.3 ml of a 0.1% or 0.15% acetone solution of DMBA on the clipped backs of mice, followed after a delay of 21 d by application of CN at a maximal tolerated concentration once or twice a week for 12-15 wk. There was a significant increase in tumors 22 wk after the start of secondary treatment.

Although CN may be a promoter, the absence of data from thorough carcinogenicity tests or from short-term mutagenicity tests makes it impossible to reach a conclusion regarding its carcinogenicity.

The National Toxicology Program performed a subchronic study of CN to generate data on the maximally tolerated dose (MTD) of this agent preparatory to launching a full-scale chronic-toxicity and carcinogenicity bioassay.[38] The test was conducted in Fischer 344

TABLE 4-23

Acute Oral Toxicity of CN and CS in Rats[a]

Solvent	Concentration of CN or CS, % w/v	Mean LD_{50} (95% Confidence limits), mg/kg[b]		LD_{50} Ratio, CS:CN
		CS	CN	
TCE	4	102 (78-134)	260 (210-322)	2.5
Carboxymethylcellulose sodium, 0.5% w/v	2[c]	71 (59-86)	358 (219-440)	5.0
Propylene glycol in water, 65% v/v	1	82(73-90)	178 (144-221)	2.2
Corn oil	2[c]	224 (184-273)	264 (234-298)	1.2
Dimethyl sulfoxide	4	52 (47-80)	318 (250-400)	6.1
TOF	2	258[d]	358[d]	1.4

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[a] Data from Gaskins *et al*.[8]

[b] Deaths occurred within 48 h of dosing, regardless of solvent. Gross abnormalities consisted of moderate to severe gastroenteritis.

[c] Suspensions.

[d] Estimated.

male and female rats and in $B6C3F_1$ hybrid male and female mice. Six groups of 20 rats and mice divided equally between sexes were exposed to a CN aerosol by inhalation at concentrations of 0 (control), 0.25, 0.5, 1.0, 2.0, and 4.0 mg/m^3 for 6 h/d, 5 d/wk for 13 wk, for a total of 65 exposures of 6 h each. In rats, reduced weight gains were seen in males at 4.0 mg/m^3 and in females at 0.25, 0.5, 1.0, and 4.0 mg/m^3. In mice, weight gain appeared normal at all concentrations, except at the last weighing. There appeared to be a slight increase in liver:body weight ratio in mice and rats at most concentrations. However, there were no gross clinical signs in rats or in mice, except those related to irritation of the eye involving opacity. Microscopically, no lesions were observed, compared with controls. On the basis of these results, it was recommended that the chronic study be conducted at 1.0 and 2.0 mg/m^3 for male and female mice and rats.

TOXICOLOGY IN HUMAN STUDIES

Immediate Effects

Exposure to CN causes an immediate burning sensation or stinging in the eyes, nose, throat, and exposed skin. Lacrimation, salivation, rhinorrhea, and dyspnea or a constricting sensation in the chest follow. The lacrimatory action persists for about 20 min after exposure, but conjunctivitis and blepharospasm may last for 24 h. CN is more toxic than CS or CR. High concentrations of CN may result in chemical injury to the eye, with corneal and conjunctival edema, loss of corneal epithelium, and chemosis.[2,20]

Acute injuries of the eyes, primarily from effects of blast and missiles, may occur from tear-gas weapons, such as pen guns. The immediate effects of these injuries include swelling and edema of the lids, with penetration of skin, conjunctiva, cornea, sclera, or globe by gunpowder and CN; conjunctival ischemia and chemosis; corneal edema, erosion, inflammation, or ulceration; and focal hemorrhage.[13,20]

Injuries in Confined Spaces

The use of CN in open spaces, as in riot control, causes copious lacrimation, rhinorrhea, and a burning sensation in exposed skin. These effects pass quickly in fresh air. A report of Thorburn[37], however, showed that results of exposure in confined spaces can be severe. In the incident described by Thorburn, 44 prison cells, each with an occupant, were sprayed with a CN aerosol, some more than once. The exhaust system did not remove the CN; some lingered in the cells as long as 4 d. After the spraying, inmates took showers while still clothed, and clothing was not changed. Twenty-eight men required medical attention; eight were hospitalized. Besides dyspnea, conjunctivitis, vomiting, allergic reactions, fainting, and pharyngitis, four men had facial burns and three developed blisters around the ankles, apparently from drainage of CN- contaminated water in clothing. One man had first- and second-degree burns over 25% of his body, and 10 men not admitted to the hospital suffered first- and second-degree skin burns. There was no serious eye injury.

Many of the serious effects on these prisoners were caused or exacerbated by a delay in treatment. It is likely that the initial spraying would have produced serious casualties even if they had not been kept in wet clothes impregnated with CN. The immediate effects of CN observed after an incident in confined spaces include lacrimation, conjunctivitis, conjunctival edema, sore throat and pharyngeal erythema, dyspnea, cough, and skin burns.[37] CN may cause death in confined spaces from which escape is not possible. If death occurs from high concentrations of CN, the postmortem examination may reveal pulmonary edema and congestion, intra-alveolar hemorrhage, necrosis of respiratory mucosa and formation of pseudomembranes, and bronchopneumonia.[6,9,25,35]

Wind-Tunnel Aerosol Tests in Man

Punte *et al.*[31] tested volunteers in a wind tunnel at an airspeed of 5 mph to establish CN tolerance time--the length of time a subject could remain in the CN-containing airstream. The tolerance time varied with the subject. CN aerosols were generated from acetone solutions and had a mass median diameter of about 0.6 µm. Such particles can reach and remain in the deep region of the respiratory tract. The immediate effects of such exposures were tingling of the nose and rhinorrhea, burning of throat and eyes, lacrimation, and blurred vision. Some subjects suffered dyspnea. Mild conjunctivitis was observed, but this and the other symptoms passed rapidly when the subject left the wind tunnel.

Effectiveness, Lethality, and Tolerance Time

The ICt_{50} and LCt_{50} of CN are 20-213 and 7,000-14,000 $mg{\cdot}min/m^3$, respectively.[25] As an aerosol, it has a TC_{50} for the eye of 0.3 mg/m^3 and an IC_{50} of 20-50 mg/m^3.[2] In field conditions, lacrimation occurs at 10 mg/m^3. The human LCt is 8,500-25,000 $mg{\cdot}min/m^3$.[2] The tentative maximal safe inhaled Ct for humans is 350 $mg{\cdot}min/m^3$.[31]

The estimated ICt_{50} of CN for man is 80 $mg{\cdot}min/m^3$, on the basis of a 1-min exposure.[25] This value is not valid for all conditions; the authors cited incapacitating concentrations of 20 and 40 mg/m^3 for 1 min under other conditions.

Estimates of the LD_{50} for humans have been based on animal data, such as shown in Table 4-23. From these and other data, official estimates of LD_{50} and safety factors have been made. A few deaths from CN poisoning have been reported, as discussed later.

TEAR-GAS WEAPONS

Forensic Aspects

The most popular weapon for personal protection against assaults and robberies over the last 20 yr is the tear-gas pen gun, a simple, inexpensive, inconspicuous device resembling a pen that carries a cartridge loaded with Mace. The load is a 1:1 mixture of CN and silicic anhydride; the propellant is smokeless powder. The cartridge is sealed with a foam rubber wad and acrylic coating.[34] The numerous injuries, especially eye injuries, inflicted by these cartridges have prompted the development of a spray with a charge of CN in 1,1,1-trichloro-2,2,2-trifluoroethane and petroleum ethers or other vehicles.[29]

Experimental Studies

Stahl *et al*.[34] tested the effects of closeup and contact shots. When a standard cartridge (with 0.26 g of CN) was fired 12 in. from the axilla of a dog, the wad caused abrasions, edema, and erythema, but did not penetrate the skin. A shot fired in contact with the skin caused a penetrating wound of the skin and axilla. Exploration of the wound 30 d later disclosed an encapsulated wad and necrosis of the axillary tissues, but no major injury to the neurovascular tissues. Another test was made by firing a magnum cartridge (with 0.7 g of CN) 6 in. from the flank of a nonanesthetized dog. This caused a nonpenetrating wound with ecchymosis of 2 cm^2. A second magnum cartridge fired in contact with the chest wall caused a large wound and almost immediate death. Muscles and pericardium were perforated, and a rib was broken.

Adams *et al*.[1] tested the effects of tear-gas cartridges fired 4 in. from the exposed sciatic nerves of six rabbits. Animals were killed for examination at intervals up to 32 d. Loss of nerve function was noted at 3-4 d. The odor of CN in the wound was noted for up to 6 d. In animals examined at 15 and 32 d, heavy scar tissue surrounded the nerves and nearby muscle. A second group of rabbits was treated by exposing the nerves and dusting them with 0.2 g of the CN mixture from a cartridge. Loss of nerve function occurred, as in the first group. All animals showed the beginning of axon breakdown on the seventh day. Adams *et al*. concluded that CN is a toxin when in contact with nerves; thus, particles of it driven into tissue by the force of a closeup shot can inflict serious injury.

Accidental Injuries

Adams et al.[1] reported three hand injuries that resulted from accidental discharges of tear-gas guns at close range. Surgery was required in all three to alleviate pain and in two to remove wadding and other foreign material. All three patients suffered continuing pain and some loss of sensation, apparently from the toxic action of CN on nerves.

Levine and Stahl[21] examined files of the Armed Forces Institute of Pathology and found records of 13 men who had lost eyes because of accidental or intentional discharges of tear-gas weapons close to the face. They emphasized the multiple nature of such wounds from the agent, wadding, and other debris. In old cartridges, the charge of CN may be clumped and thus increase entry of CN into tissue.

Many other reports of eye injuries have been published. Hoffman[13] recommended outlawing all tear-gas weapons. Oksala and Salminen[27] reported six cases, Hoepping[12] reported 20 cases of keratitis and other ocular injuries from pen guns, and Laibson and Oconor[16] treated five cases of eye injury. Oaks et al.[26] urged that police officers be instructed to handle tear-gas guns properly and, when prisoners are in pain after gassing, to get medical attention promptly. One patient shot in the face at close range with a tear-gas gun was imprisoned without medical treatment for 44 h; his sight took a month to recover.

SENSITIZATION IN HUMANS

CN is a skin sensitizer. Rothberg's demonstration of this in guinea pigs has ample confirmation in clinical reports of sensitization in man.[33] Penneys et al.[29] reported treating two patients for allergic contact dermatitis, each having had two or more exposures to the agent. In the course of treatment, one patient was given a patch test with Mace (a formulation containing CN), the other a patch test with CN. A patch test with Mace was applied to one investigator's skin, and one with CN to the skin of another. All four test subjects had positive reactions. Penneys et al. applied 0.009% CN (0.01 the concentration found in Mace) to 30 nonexposed volunteers. Eight of these subjects were then given patch tests with 0.25 ml of 0.9% CN for 24 h. Five of the eight developed allergic contact sensitivity to the agent. They were challenged with 0.009% CN 2 and 3 wk later. Erythema, edema, and vesiculation developed at the test area. Using these subjects, Penneys et al. demonstrated cross-sensitization to 1,1-dichloroacetophenone, but not to 1-bromoacetophenone, p-chloroacetophenone, or acetophenone. Maibach and Marzulli[23] showed that CN is a potent contact sensitizer with a capacity for producing cross-reactions with CS.

Penneys et al.[29] tested the speed of action of CN on skin. They applied Mace to the forearms of three sensitized subjects in five spots. The Mace was then washed off with soap and water at 30 s or 1, 2, 3, or 4 min. There were no reactions to a 30-s exposure, one at 1 min, two at 2 min, and three at 4 min. In a riot, when CN is used, no rioters would be expected to remove the agent quickly by washing, and any who had a previous exposure to Mace or CN might suffer dermatitis.

CN may cause primary irritant dermatitis or allergic contact dermatitis by delayed hypersensitivity. After sensitization, acute exposure to CN causes itching, erythema, edema, vesiculation, purpura, and necrosis.[28] Jolly and Carpenter[14] reported that an accidental discharge of a pen gun resulted in erythema and edema 24 h later; the patient had been exposed to CN 5 yr earlier. Queen and Stander[32] reported severe reactions to CN 17 yr after a first exposure to the agent.

LONG-TERM EFFECTS

Acute eye burns caused by discharge of tear-gas weapons at close range have been reported. In these instances, the charge and seal, as well as the CN, penetrated the tissues, thereby causing mechanical damage.[13] However, gross contamination of the eyes with CN can also result in severe and permanent corneal injury.[10] CN is thought to act on the eye by two distinct mechanisms. In one, it exerts a lacrimatory, reversible, and essentially noninjurious effect on corneal nerve endings at low concentrations; in the other, it causes an injurious, denaturing reaction on the nerve endings (reversible only with difficulty), probably with other components of cornea and conjunctiva affected at high concentrations.[10] CN induces contact dermatitis,[28] but it is not known whether this leads to persistent skin or respiratory problems as reported in animals for toluene diisocyanate.[15]

EFFECTS ON HUMAN SUBJECTS AT EDGEWOOD

Between 1958 and 1972, 99 human subjects underwent experimental exposures to CN at Edgewood Arsenal. Sixty-nine subjects had aerosol exposures in a chamber that they entered masked; they removed the masks after the agent concentration had equilibrated. Thirty subjects had direct skin applications of CN.

Exposure data are available on 68 of the 99 subjects: 16 had one to five aerosol exposures in 1958, 44 had one to three aerosol exposures in 1965, and eight had one dermal application in 1968. No exposure data are available on the other 31 subjects: nine had one aerosol exposure and performed simulated battlefield functions in 1966, five had one dermal application in 1967, and 17 had one dermal application in 1972.

The only dosage information on the sixteen 1958 aerosol-exposure subjects is exposure time, which was recorded on seven of the subjects and ranged from 0.32 to 3.63 min. The 44 subjects who underwent aerosol exposures in 1965 experienced Ct's of 6-315 $mg \cdot min/m^3$ and exposure time of 0.15-3 min. The eight dermal- exposure subjects in 1968 had 0.01-0.025 ml of CN applied to their bare or clothed arms.

The effects on the aerosol-exposure subjects were transient, generally resolving within minutes of removal of the agent. There also seemed to be tolerance in experienced subjects, often increased by closing the eyes. Predominant effects of aerosol exposure were ocular: lacrimation, blepharospasm, conjunctivitis, and, rarely, palpebral edema. Respiratory effects of aerosol exposure were nasopharyngeal irritation, rhinorrhea, and, rarely, dyspnea. Skin irritation was prominent on shaved areas. Other rare effects of aerosol exposure were headaches and dizziness. No laboratory analyses were recorded for aerosol exposure.

Only one of the eight 1968 dermal-exposure subjects had erythema at the exposure site, which lasted for 7 h. The CN was applied to his skin. Five of the 1968 dermal-exposure subjects had normal results of laboratory analyses--including urinalysis, complete blood count, blood urea nitrogen, alkaline phosphatase, and serum glutamic oxalotransferase--7 d after exposure.

In summary, among the 68 subjects from Edgewood on whom there are data, there were probably no permanent ocular or pulmonary injuries. These were short, low-dose exposures, and effects on the eyes and respiratory system were transient; symptomatic recovery was complete within minutes. Information on the dermal effects of CN exposure is minimal. Sensitization to CN is likely, causing allergic contact dermatitis and possible systemic allergic reactions (e.g., pulmonary fibrosis) on re-exposure, although there is no evidence that this occurred among the Edgewood subjects.

DISCUSSION

It has been suggested that reactions of CN with sulfhydryl groups may be involved in the toxic effects of CN.[2,7,11] Reaction with nucleophilic centers (e.g., sulfhydryl groups) at the binding or catalytic sites of enzymes may lead to enzyme inhibition.

Castro[5] investigated the inhibitory actions of several alkylating agents on cholinesterase (ChE) and found that CN was "an instantaneous and noncompetitive inhibitor." The inhibition of ChE was reversible by dilution or dialysis. Castro concluded that inhibition of ChE by CN does not involve the sulfhydryl group, but possibly the histidine or methionine groups of the enzyme. He suggested that the rapid and reversible ChE inhibition by CN might explain the rapid onset of lacrimation and the correspondingly quick recovery when the subject moves to clean air.

SUMMARY

- CN is moderately toxic (more so than CS), but nonlethal, except at high dosage. There appear to be no lasting effects on eyes or skin of Edgewood subjects after single or multiple aerosol exposures.

- CN was found to be active as a promoter in one study. Adequate tests have not been conducted, however, to permit conclusions about the carcinogenicity of CN itself. Similarly, the available information permits no conclusions with regard to mutagenicity. Studies now being conducted by the National Toxicology Program should provide more information on these subjects.

- CN is a strong sensitizing agent, often producing allergic contact dermatitis after a single exposure. Edgewood subjects exposed to aerosolized CN might now be skin-sensitized and at greater risk of hypersensitivity to inhaled CN, if effects on CN parallel those of toluene diisocyanate on animals in this regard.

- The use of CN-loaded (Mace-loaded) pen guns and pistols presents severe hazards, often not caused by the agent itself, because of improper handling and accidental discharge.

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DIBENZ[b,f][1,4]OXAZEPINE

CHARACTERISTICS

Dibenz[b,f][1,4]oxazepine (CR) is a pale yellow solid, with a molecular weight of 195.2 and a melting point of 72.5°C (Table 4-1). It is slightly soluble in water (3.5×10^{-4} mol/L at 20°C), but freely soluble in most organic solvents.[2]

Bandman and Savateyev[8] and Choie and Landis[10] published recent reviews on CR.

CYTOTOXICITY

Lee and Webber[14] studied the toxicity of CR in HeLa cells in culture. In vivo experiments had disclosed only negligible effects on tissue cells, except for the sensory elements of the nervous system. Lee and Webber believed that suitable cell cultures might react to compounds of moderate toxicity, thus constituting sensitive tests that would indicate which cellular elements were attacked by compounds like CR.

CR was added to cell cultures at various concentrations. The agent was washed out, cultures were incubated for 7 d, and effects of CR were judged in terms of changes in the morphology of the cells. After exposure at 4×10^{-5} mol/L for 3.5 h, there was no change in cell morphology. After exposure at 1.4×10^{-4} mol/L for 1.75 h, there was cytoplasmic shrinkage and increased staining of cell membranes. Cells with aggregations of heavily stained blebs, usually surrounding the nucleus, were about 20% of the population. After exposure at 1.4×10^{-4} mol/L for 3.5 h, the same alterations were seen, but in greater numbers. In the growth inhibition test, ranging tests yielded a GID_{50} (inhibition of growth for 50% of the population) of 10^{-4} mol/L for CR. A second series of tests with a narrow range of concentrations gave a final value of 8.7×10^{-5} mol/L. The authors speculated that results with lacrimators are based on interference with sulfhydryl-containing enzymes.

Upshall[21] exposed pregnant rats and rabbits to CR at several doses and by several routes of administration to test for teratogenic or embryolethal effects. Inhalation exposure times were 5-7 min for rats and rabbits. Other conditions were as follows: Rats and rabbits were exposed to aerosolized CR at Ct's 10,000 and 1,000 on days 6-15 or 6-18 of pregnancy, respectively. Other rats were exposed to CR by intragastric administration at 2, 20, and 100 mg/kg on days 6, 8, 10, 12, and 14 of pregnancy. Other rats were exposed at 400 mg/kg CR intragastrically on day 7, 10, or 13 of pregnancy. Rabbits were exposed to CR by intragastric administration at 0.2, 2, and 20 mg/kg on days 6, 8, 20, 12, 14, 16, and 18 of pregnancy or intravenously at 14.1, 15.8, or 17.8 mg/kg on selected days of pregnancy. Recorded results included number of litters, number of live fetuses, litter size and weight, placental weight, increase in placental weight, and number of abnormal litters. High inhalation and intragastric doses of CR were not shown to be teratogenic, but the selected exposure concentrations did not reach maternally toxic values. Intravenous administration to rabbits at a dose close to the maternal LD_{50} caused fetal deaths. The author concluded that CR is neither teratogenic nor embryolethal to rats and rabbits when given by inhalation or intragastric intubation. He did not offer a satisfactory resolution of the rabbit-injection studies.

TOXICITY IN ANIMALS

Acute Effects

CR is a potent peripheral sensory irritant of low toxicity by the usual routes of administration.[3] It appears safer than CS, which replaced CN and DM in turn as riot-control agent because of greater effectiveness and lower toxicity. Table 4-20 shows comparative toxicities of these compounds in several species.[2]

Ballantyne[3] studied the toxicity of CR in several species of laboratory animals (Table 4-24). CR was more toxic to rabbits and rats than to mice by single-dose intravenous injections. There were no sex-related differences for rats and mice. Signs of poisoning developed in seconds. Survivors appeared normal in 1 h. Animals that died did so in 4-10 min, with congestion of alveolar capillaries and liver sinusoids on autopsy examination. Survivors, sacrificed for autopsy 15 d later, exhibited no abnormalities. Toxic signs appeared in 2-5 min after single-dose intraperitoneal injection, with deaths 1-4 d after injection. Survivors recovered in about 24 h. Animals that died showed congestion of alveolar capillaries, some hemorrhage, and congestion of liver, kidneys, and small intestine. Survivors, examined 14 d later, showed no abnormalities. Survivors of orally administered CR showed ataxia and weakness for 1-2 d, then appeared normal. Animals that died did so in 1-6 d, with congestion of gastric and intestinal mucosa, liver, and lungs and renal tubular necrosis. Survivors were normal when sacrificed for necropsy. Tables 4-25, 4-26, and 4-27 summarize results of inhalation experiments. Few deaths occurred, even at the highest exposures. Rats, rabbits, guinea pigs, and mice were exposed to smokes from grenades, both with and without CR. CR contributed very little to the toxic effects of grenade smokes. The toxicities of CR, CS, and CN are compared in Table 4-28.

Owens *et al.*[17] and Biskup *et al.* [9] studied the effects of 1% solutions of CR in propylene glycol, dipropylene glycol, diethylene glycol monomethyl ether, and propylene glycol/water solutions. These solutions were tested in guinea pigs, rats, rabbits, dogs, and monkeys. Doses were administered to the eyes (0.2 ml) and skin (1.0 ml) and by intratracheal insufflation (0.5 ml). No test revealed residual damage. The intent of these studies was to assess the effects of the solvents as carriers of CR.

TABLE 4-24

Acute Oral and Parenteral Lethal Toxicity of Solutions of CR in Various Small Mammals[a]

Route	Species	Sex	LD_{50} (95% Confidence Limits), mg/kg
Intravenous	Mouse	Male	130 (101-167)
	Mouse	Female	112 (103-121)
	Rat	Male	68 (60-77)
	Rat	Female	68 (61-76)
	Rabbit	Female	47 (42-68)
Intraperitoneal	Rat	Male	817 (747-1,007)
	Rat	Female	766 (719-818)
	Guinea pig	Female	463 (337-607)
Oral	Mouse	Female	4,000
	Rat	Male	7,500 (6,400-12,100)
	Rat	Female	5,900 (5,100-8,500)
	Rabbit	Female	1,760 (1,350- 2,400)
	Guinea pig	Female	629 (555-712)
Percutaneous	Rabbit	Female	450 (in corn oil)
	Rabbit	Female	400 (in petrolatum paste)
	Rabbit	Female	1,500 (in DMSO)

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[a] Data from Ballantyne[3]

TABLE 4-25

Exposure of Rats to CR Aerosols[a]

Duration of Exposure, min	CR Concentration, mg/m^3		Mean Ct, $mg \cdot min/m^3$	14-Day Mortality, no. deaths/no. exposed
	Average	Range		
15	870	780-965	13,050	0/5
30	960	900-1,140	28,800	0/5
60	970	860-1,050	58,200	0/5
60	1,370	1,220-1,570	82,200	0/5
70	1,360	1,070-1,500	95,200	0/5
84	1,230	910-1,740	103,320	0/5
80	1,470	1,120-1,680	117,600	0/5
120	1,650	1,200-2,060	198,000	0/5
123	2,030	1,770-2,200	249,690	0/5
180	2,380	2,300-2,520	428,400	0/5

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[a] Data from Ballantyne[3]

TABLE 4-26

Exposure of Rabbits, Guinea Pigs, and Mice to CR Aerosols[a]

Duration of Exposure, min	CR Concentration, mg/m^3		Mean Ct, mg·min/m^3	14-Day Mortality, no. deaths/no. exposed		
	Average	Range		Rabbits	Guinea pigs	Mice
14	670	480-1,210	9,380	0/20	0/20	0/40
120	490	350-620	58,800	0/5	0/5	0/20
120	570	550-610	68,400	0/10	0/10	0/20
193	690	530-880	133,170	0/10	1/10	0/20
113	1,500	1,290-1,930	169,500	9/20	5/20	2/40

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[a] Data from Ballantyne[3]

TABLE 4-27

Exposure of Hamsters to CR Aerosols[a]

Duration of Exposure, min	CR Concentration, mg/m^3 Average	CR Concentration, mg/m^3 Range	Mean Ct, mg·min/m^3	14-Day Mortality, no. deaths/no. exposed
60	710	610-800	42,600	0/10
220	1,060	1,000-1,180	233,200	0/10
200	1,850	1,310-2,030	370,000	1/10
180	2,450	2,350-2,680	441,000	2/10

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[a] Data from Ballantyne[3]

TABLE 4-28

Comparative Single-Dose Lethal Toxicity of CN, CS, and CR[a]

		LD_{50} (95% Confidence Limits)[b]		
Route	Species (Sex)	CR	CS	CN
Inhalation (smoke)	Rat	139,000 (119,000-157,000)	68,000 (60,000-77,000)	23,000 (20,000-27,300)
	Rabbit	160,000 (134,000-188,000)	63,000 (50,000-80,000)	15,800 (8,700-28,600)
	Guinea pig	169,000 (139,000-201,000)	35,000 (25,000-45,000)	15,400 (11,300-21,100)
	Mouse	203,000 (180,000-229,000)	76,000 (61,000-119,000)	
Inhalation (aerosol)	Rat	428,400	88,460 (77,400-98,500)	3,700-18,800[c]
	Rabbit	169,500	54,100 (42,630-70,400)	5,840-11,480[c]
	Guinea pig	169,500	50,010 (42,750-60,220)	3,500-13,140[c]
	Mouse	169,500	67,200 (59,200-78,450)	18,200-73,500[c]
Intravenous	Mouse (male)	130 (101-167)	48 (41-63)	81 (66-109)
	Mouse (female)	112 (103-121)	--	--
	Rat (male)	68 (60-77)	--	--
	Rat (female)	68 (61-76)	28 (25-30)	40 (34-49)
	Rabbit (female)	47 (42-68)	27 (25-30)	29 (16-39)
Intraperitoneal	Rat (male)	817 (747-1,007)	48 (43-54)	38 (31-54)
	Rat (female)	766 (719-818)	--	--
	Guinea pig (female)	463 (337-607)	73 (62-78)	17 (8-21)
Oral	Mouse (female)	4,000	--	--
	Rat (male)	7,500 (6,400-12,100)	1,366 (1,184-1,780)	126 (113-144)
	Rat (female)	5,900 (5,100-8,500)	1,284 (1133-1530)	--
	Rabbit (female)	1,760 (1,350-2,400)	142 (61-236)	118 (93-143)
	Guinea pig (female)	629 (555-712)	212 (190-243)	157 (122-229)

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[a] Data from Ballantyne[3]

[b] In mg·min/m^3 for smokes and aerosols; in mg/kg for intravenous, intraperitoneal, and oral routes.

[c] Range of values from several sources.

Ocular Effects

Rengstorff et al.[19,20] supplemented the ocular exposures of Owens et al.[17] by testing the effects of a 5% solution of CR in propylene glycol in the rabbit eye. Owens et al. had instilled 0.2 ml of 1% solutions of CR in propylene glycol, dipropylene glycol and diethylene glycol, monomethyl ether in single doses and on 5 successive days. Neither rabbits nor monkeys suffered more than mild and transitory eye effects.

Rengstorff et al.[20] applied 0.025 ml of a 5% solution of CR 5 d/wk for 4 wk. The animals were kept under observation for 60 d. Instillation of the daily doses was followed by a brief period of blepharospasm (about 15 min), after which the eyes appeared to be normal. During the test period, superficial and slit-lamp examination did not reveal any injury to the eyes. At the end of the experimental period, the eyes were examined by light and electron microscopy. No abnormalities were found.

Ballantyne et al.[6] conducted extensive investigations of the effects of CR on the eyes of rabbits. Similar tests were applied with CN.

Eye effects were as follows:

- Lacrimation: very mild at 1 and 2% for 1 h, mild at 5 and 10% for 2-3 d.
- Blepharitis: mild at 2% for 24 h, up to 2 d at 5 and 10%.
- Chemosis: mild to moderate at 5 and 10% for 2 d.
- Hyperemia: mild at 1 and 2% for 48 h, moderate at 5 and 10% for up to 3 d.
- Iritis: mild in 2 of 10 rabbits at 5%, in 3 of 10 at 10%.
- Keratitis: mild at 10% for 21 d, at 5% for 3 d.
- Effects of solvents: less severe than with CN for all solvents.
- Solid CR: 5 mg dropped into the eye caused minor lacrimation, blepharitis, and chemosis, clearing in 1 h.
- CR aerosols: 30-min exposures at 10,200 mg·min/m^3 and 17,130 mg·min/m^3 resulted in only mild lacrimation and conjunctival injection, clearing in 1 h.

- Corneal thickness: increase in thickness was dose-dependent, and cornea returned to normal in 2 d after CR at up to 2%, in 13 d after CR at 10%.

- Intraocular tension: CR at 0.5-5% caused increases of 6-40%, which subsided in 1 h.

Ballantyne et al. concluded that these experiments demonstrated a much greater degree of safety for CR and less (almost nonexistent) damage to the eye than CN. Colgrave et al.[11] examined the fine structure of exposed rat lungs by optical and electron microscopy after exposure to CR aerosols at high concentrations, as follows:

Concentration, g/m^3	Time, min	Ct,$\times 10^3$, $mg{\cdot}min/m^3$
1.15	68	78
1.22	115.5	141
1.02	158	161

No animals died from these exposures. Examinations of the lung tissues showed mild congestion, some capillary damage in the form of endothelial ballooning, and some endothelial swelling and thickening. These effects were judged reversible.

Lung Effects

Pattle et al.[18] exposed 10 rats to a CR aerosol, generated pyrotechnically, at 115,000 $mg{\cdot}min/m^3$ for 1 h. The lung surfactant and organelles from which it appears to be derived showed no changes up to 15 d after exposure, when examined by light and electron microscopy.

Metabolism and Physiology

Balfour,[1] using tritium-labeled CR to investigate uptake and metabolism, found that both intact cornea of the guinea pig and corneal homogenates take up CR readily and metabolize it to a lactam derivative. The metabolic process appeared to take place in the corneal cells. Homogenization of the corneas supported this idea: after homogenization, the metabolic activity was found in supernatant fluid. The author speculated that the lactam derivative of CR is not involved in the process of irritation, but that methyl derivatives are involved.

Lundy and McKay[15] studied the effects of intravenous CR on cardiovascular activity in anesthetized cats. Dose-dependent stimulation of heart rate and blood pressure of short duration was seen, with increased arterial catecholamine content. Blood pressure increased by about 5% after a dose of CR at 25 mg/kg and about 60% after 200 mg/kg. The brevity of the increase was attributed to rapid metabolism of CR. Pretreatment with phentolamine completely blocked the increase in blood pressure. Pretreatment with propranolol almost completely blocked the tachycardia. Cats pretreated with 6-hydroxydopamine did not show cardiovascular stimulation. Bilateral adrenalectomy did not alter the cardiovascular response to CR. The pressor effect of CR was attributed to release of norepinephrine from adrenergic nerve endings.

Additional studies of Lundy and McKay[16] suggested that the CR-induced increases in heart rate and blood pressure in the cat are mediated by the sympathetic nervous system.

Lundy and McKay were aware of the report of Ballantyne et al.[5] describing the cardiovascular changes (decrease in heart rate and increase in blood pressure) that follow "splash contamination" of the human face (no details given). The results of their experiments on the cat heart suggested to Lundy and McKay that the cardiovascular effects described by Ballantyne et al. could be explained by the absorption of enough CR to produce a systemic effect on the heart via the sympathetic nervous system. The second paper of Lundy and McKay, discussing the splash effect, appeared in 1977. However, in the 1976 report of Ballantyne et al.,[5] the possibility of systemic effects of absorption was rejected, because the authors concluded that, even with a whole-body "drench," not nearly enough CR could be absorbed fast enough to cause the immediate increase in blood pressure observed in their experiments. They did not refer to work of Lundy and McKay, which appeared around the same time.

Leadbeater and Maidment[13] studied the absorption of CR given to rats as an aerosol, by gavage, or by intravenous injection. Absorption from the aerosol was measured by scintillation counting of the ^{3}H-labeled CR. Both ^{3}H and ^{14}C labels were used for other experiments. Absorption and metabolism were rapid. Radioactivity derived from the CR aerosols was detected in the blood within 15 s of the beginning of exposure. The plasma half-life of CR given intravenously was about 5 min; the half-life of the CR absorbed through the lungs was about the same. CR and its metabolites were found in the blood within 10 min after intragastric administration. The immediate metabolic products were not identified, but glucuronides were identifiable later. Leadbeater and Maidment, like Lundy and McKay, believed that the rapid absorption of CR may imply a systemic involvement in its physiologic effects.

TOXICITY IN HUMANS

Ocular Effects

Ballantyne and Swanston,[7] applying procedures developed with CS, measured threshold concentrations of CR in saline solution for producing blepharospasm: in the rabbit, 7.9×10^{-5} M; in the guinea pig, 3.5×10^{-5} M; and in man, 8.6×10^{-7} M. Threshold concentrations for sensation were 4.9×10^{-7} M in the human eye and 2.1×10^{-6} M in the human tongue. The authors cited data from which a CR threshold concentration of 4×10^{-3} mg/m^3 of air was calculated. The threshold concentration of solutions in the eye is 4.9×10^{-7} M, or 4.1×10^{-2} mg/L of solution. The corresponding value for aerosols is 4×10^{-6} mg/L of air, so the human eye is much more sensitive to CR aerosols than to solutions. From the data in Table 4-29, it can be seen that, for the rabbit and guinea pig, CS is more potent than CR at the threshold concentration. For the human eye and tongue, however, CR is more potent. Caution must be used in extrapolating data from animals to humans. The authors calculated a safety factor of several thousand between the highest concentration used in these threshold-concentration tests and the 5% (0.256 M) concentration that is the lowest likely to produce just-detectable damage to the eye.

Cutaneous Effects

Weigand and Mershon[22] tested 39 subjects for skin reactions to CR, using 1-cm^2 patches soaked with CR-propylene glycol solutions. Each patch was wetted with 0.1 ml of 0.01, 0.05, 0.10, 0.25, 0.50, or 1.0% CR. Exposure times were 5 and 30 min, and tests were made at 18.3 and 25.6°C. The concentration of CR did not affect the time of onset of sensation; higher temperature decreased onset times. Subjects differed widely, both in time of onset and termination of irritation and in reported intensity of sensation. Intensity was not related to concentration of CR, exposure time, or temperature. The degree of erythema was variable, but it disappeared in 2-4 h. All CR concentrations were judged to be relatively harmless.

Holland[12] tested skin reactions to CR by putting measured amounts of CR powder on the skin sealed under 4-cm-diameter watch glasses. Some were dry, others wetted with 2 drops of saline solution. After a 1-h exposure, the CR was removed and the skin was washed. Results are presented in Table 4-30. Erythema appeared in 10 min and faded out by 30 min after removal of the CR. No swelling or vesication developed, and no residual skin changes were seen. In comparing the results with those of similar tests of CS and CN, Holland commented that all reactions to CR are transient and mild, whereas CS causes longer-lasting erythema and CN causes blistering.

Ballantyne et al.[4] have given a general description of the effects on humans of dilute solutions of CR in a water-polyethylene glycol mixture as a whole-body spray. Their report was intended mainly as a compendium of symptoms with recommended procedures for treatment, if needed. A detailed report on the effects of these "drenches" was published by Ballantyne et al.[5] They examined the effects of CR and CS when subjects were given either showers or sprays from a hose, as might be experienced by rioters if a water cannon were used. CR solutions for the drenches were made up with 3.3% (v/v) dipropylene glycol monomethyl ether as a cosolvent. Control tests were run with water alone and water plus dipropylene glycol monomethyl ether. The control drenches, like other cold showers, caused a mild shock and a transient increase in blood pressure.

CR-containing drenches were tested at 0.001% and 0.0025% for both individuals and groups. Within a minute after these drenches, the subjects felt an intense stinging of the eyes, with blepharospasm. Then the facial skin began to sting; this increased to a strong burning sensation. Lacrimation, rhinorrhea, and salivation also occurred. In another minute, the stinging and burning spread to the neck, shoulders, back, and genitalia. Other parts of the body were less strongly affected. After 15-20 min, sensation deceased to a mild tingling. A mild erythema of the skin, lasting 1-2 h, developed.

Physiologic Studies

All the subjects in the studies of Ballantyne et al.[4] were monitored for changes in blood pressure. CR drenches caused an immediate increase in blood pressure. A mean increase in systolic pressure of 45 mm Hg was observed after drenches with 0.001% CR, and a mean of 59 mm Hg after drenches with 0.0025% CR. Corresponding increases in diastolic pressure were 23 and 29 mm Hg. Increases above control values were highly significant, but the mean peak increases for the two concentrations did not differ significantly. Increases in blood pressure lasted about as long as the sensory irritation. Concomitant with the increase in blood pressure, Ballantyne et al. observed nonuniform changes in heart rate. The first report[4] stated that "heart rate in the majority of subjects

TABLE 4-29

Comparison of Threshold Irritant Response of Eyes of Guinea Pig and Rabbit and Eyes and Tongue of Man to CR Solutions[a]

			Threshold Concentration (95% Confidence Limits		
Species	**Organ**	**Response**	**CR**	**CS**	**Potency Ratio, CR:CS**
Guinea pig	Eye	Blepharospasm	3.5 (2.8- 4.3) x 10^{-5} M	2.2 (1.9- 2.4) x 10^{-5} M	0.63:1
Rabbit	Eye	Blepharospasm	7.9 (5.1-12.5) x 10^{-5} M	5.9 (3.8-10) x 10^{-5} M	0.75:1
Man	Eye	Blepharospasm	8.6 (6.8-12.5) x 10^{-7} M	3.2 (2.1- 6.1) x 10^{-6} M	3.7:1
Man	Eye	Sensation	4.9 (3.8- 6.5) x 10^{-7} M	7.3 (4.2-11.2) x 10^{-7} M	1.5:1
Man	Tongue	Sensation	2.1 (0.7- 3.1) x 10^{-6} M	6.8 (5.0-10.6) x 10^{-6} M	3.2:1
Predicted minimal incapacitating concentration (by log-probit plot)			3.3 x 10^{-6} M	2.7 x 10^{-5} M	8.2:1

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[a] Data from Ballantyne and Swanston.[7]

TABLE 4-30

Numbers of Subjects with Reactions Produced by CR Applied to Skin of Forearm[a]

Amount of CR, mg	Reactions			
	Dry		Wet	
	Irritation	Erythema	Irritation	Erythema
0.5	--	--	+4/4	+4/4
1.0	--	--	+4/4	+4/4
2.0	-0/2	+2/2	+6/6	+6/6
5.0	+3/6	+3/6	+6/6	+6/6
10.0	+3/6	+3/6	+6/6	+6/6
15.0	+1/2	+2/2	++2/2	++2/2
20.0	+2/2	+2/2	+++2/2	+2/2
25.0	+1/6	+1/6	+++5/6	++4/6

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[a] Data from Holland.[12]

[b] -, negative; +, mild; ++, moderate; +++, severe.

usually falls." The second report[5] noted that "pulse rates increased in all subjects but with marked individual variations." "Five subjects out of the 13 had a relative bradycardia during the first few minutes after drenching, after which pulse rates increased." Ballantyne et al. appear to have considered the changes in blood pressure as much more important than the rate changes. Exercise after the drenches did not affect the increase in systolic blood pressure, but the diastolic pressure decreased. The authors believed that the increase in blood pressure was brought about by the irritant effect of the agent, and not by a cold pressor effect or by absorption of CR to produce a pharmacologic action. Because exercise after the drench did not add to the increase in blood pressure, it was concluded that the CR drench presented no more of a hazard than exercise alone.

EFFECTS ON HUMAN SUBJECTS AT EDGEWOOD

From 1963 to 1972, 97 subjects underwent experimental exposures to CR at Edgewood: 33 had aerosol exposures in a chamber, and 64 had cutaneous exposures.

In 1963, four subjects had aerosol exposures of 4.8-34 $mg \cdot min/m^3$ (duration, 17.5 s to 1 min). In 1967, 29 subjects had aerosol exposures of 0.01-1.7 $mg \cdot min/m^3$ (duration, 1 s to 6.75 min); one had two exposures.

In 1969, 24 subjects had open patch tests with 0.1 or 0.25-1.0% CR applied to their foreheads, faces, necks, and hands for 5 or 30 min, and 20 had closed patch tests with 0.01, 0.1, or 0.25-1.0% CR applied to their foreheads for 5 or 30 min.

In 1972, 11 subjects wearing protective goggles in the wind tunnel had CR sprayed on their faces from 3 or 12 ft for 10 s. The exposed areas were then washed. Concentrations of the CR sprays are not available. Also in 1972, nine subjects had cutaneous exposures to CR and CN on the same day; other data on these subjects are not available.

Several CR subjects exposed in the aerosol chamber commented that CR was milder than "tear gas." The effects of exposure to CR aerosol were transient and predominantly respiratory and ocular. All subjects with aerosol exposures had upper respiratory tract irritation with choking. Many subjects had dyspnea; some were tachypneic. Ocular irritation from aerosol exposure was common, often causing blinking, closing of the eyes, and lacrimation and interfering with the subject's ability to carry out simulated battlefield functions.

The effects of CR in the open and closed patch applications were stinging and erythema at the exposure sites, which resolved by 24 h after exposure. Applying the CR under a Telfa patch did not worsen the effects.

The effects of skin sprays in the wind tunnel included skin irritation at the exposure site, sometimes accompanied by erythema; lacrimation and conjunctivitis; upper respiratory tract irritation; and, rarely, numbness at the exposure site. The effects generally began after the exposure and worsened initially. Two wind-tunnel subjects said the effects of CR were similar to or worse than those of CS.

Many of the aerosol and wind-tunnel subjects had laboratory analyses 7 d after exposure. There were no abnormalities in the results that had not been present in pre-exposure analyses.

Given the available data on short-term exposures of Edgewood subjects to CR, as well as literature data, it is not possible to predict whether long-term health effects will result from the CR exposures.

SUMMARY

CR has been studied as a possible replacement for CS. CR has a lower mammalian toxicity than either CN or CS. Its effects are in general the same as those of CS, but they could be achieved with substantially lower quantities of agent.

Not only is the acute toxicity of CR extremely low, with an estimated human LCt_{50} over 100,000 $mg \cdot min/m^3$, but the overt signs of exposure are even more transitory than those of CS. Eye irritation passes in 15-30 min, and skin irritation in 15-20 min. Erythema, which develops only on contaminated skin, passes in about an hour and does not lead to vesication or to contact sensitization. The abrupt increase in blood pressure, which has been observed after whole-body drenches of CR in solution, subsides rapidly. Although available results show no long-term health effects of exposure to CR, there are no available data on the mutagenicity and carcinogenicity of this compound, and the data on teratology are limited.

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DIPHENYLAMINOCHLORARSINE

CHARACTERISTICS

Diphenylaminochlorarsine (DM) is a canary-yellow crystalline solid (see Table 4-1). It is insoluble in water, but soluble in organic solvents; it melts at 195°C and boils at 410°C.[5] It was first prepared by Weiland in Germany (1915) and independently (1918) by Adams in the United States. During and after World War I, it was known as Adamsite.[1] Sim[10] described its odor as that of burning fireworks. DM can be disseminated as an aerosol, either from pyrotechnic mixtures in grenades or bombs, sprayed as a solution, or dispersed as a dust. It has been described as an irritant smoke, a sternutator, and a "sneeze gas."

TOXICITY IN ANIMALS

No reports were found that provide information on whether DM is mutagenic, carcinogenic, or teratogenic.

Owens et al.[5] conducted extensive investigations of the incapacitating effects of DM in humans and its toxicity in several species of laboratory animals. McNamara et al.[4] summarized this work in 1969. The information given here is based on both these reports, which were the outcome of a request from the President's Science Advisory Committee. It was apparently believed that previous research on DM, done mainly soon after World War I, was insufficient and unsatisfactory because of the variability in the methods used and in agent purity. Furthermore, because all the early work had been done with DM dispersed in laboratory tests, it was decided to test the results of dispersion from munitions.

The immediate responses of various species of animals to DM are as follows:

- Mice, rats, and guinea pigs: The animals become hyperactive at once. In a few minutes, lacrimation and salivation begin. Hyperactivity gives way to lethargy, with labored breathing. This state may persist for 1-2 h after removal from the exposure chamber. Survivors return to normal in about 2 wk.

- Rabbits: The animals exhibit ocular and nasal irritation, hyperactivity, dyspnea, and convulsions.

- Dogs: Hyperactivity, jumping, and barking are the first signs; they are followed by salivation, retching, and vomiting. The dogs become atactic and, after removal from the chamber, hypoactive. Gagging and vomiting persist for 24 h. Little food or water is consumed for a week, after which survivors return to normal.

- **Monkeys**: During exposure, salivation, rhinorrhea, dyspnea, vomiting, and ataxia are seen. After exposure, lethargy, coughing, and vomiting persist for 24-48 h.

- **Goats**: During exposure, the animals are hyperactive, shaking their heads, rearing up, frothing at the mouth, then becoming atactic. Survivors are hypoactive and gag and vomit for a week.

- **Swine**: During exposure, the animals have dyspnea, salivation, frothing at the mouth, and ataxia. Survivors have dyspnea, loss of weight, and dehydration.

McNamara *et al.*[4] reported LCt_{50}s in seven species of animals exposed to DM dispersed in 10% acetone solutions. The combined LCt_{50} was 12,305 mg·min/m^3 (range, 10,283-14,726) (Table 4-31).

Owens *et al.*[5] exposed monkeys, dogs, and guinea pigs on 10 consecutive days to DM aerosols from a No. 113 grenade. The mean daily exposures were at about the LCt_5 in one set of animals and at the LCt_{20} to LCt_{25} in another set. In both sets, the accumulated uptake should have killed all the animals if given in a single exposure. The low concentration killed five of eight monkeys. At the high concentration, slightly more monkeys and guinea pigs and fewer dogs than would have been expected died from a single exposure. There was little indication of cumulative toxicity. Exposures and mortality are in Table 4-32.

Punte *et al.*[7] summarized their observations on mice, rats, and guinea pigs and stated that animals killed (or dying) after exposure to DM aerosol at Ct's greater than 500 mg·min/m^3 showed hyperemia of the trachea, pulmonary congestion, edema, and pneumonia. At less than 500 mg·min/m^3, no pathologic changes were observed.

Striker *et al.*[12] conducted a study of the effects of DM dispersed from No. 113 grenades. Thirty *Macaca mulatta* monkeys in groups of 10 were exposed as follows:

Average DM Concentration, mg/m^3	Duration of Exposure, min	Ct, mg·min/m^3
855	3	2,565
1,708	5	8,540
2,615	11	28,765

The animals were killed and examined at 12 and 24 h and 3, 7, and 30 d. At the low exposure, one animal examined 12 h after exposure had superficial tracheitis, edema of the tracheal and bronchial mucosa, and beginning bronchorrhea. All others were free of lesions related

TABLE 4-31

Acute Inhalation Toxicity of DM Disseminated from a 10% Acetone Solution[a]

Species	Bliss-Calculated LCt$_{50}$, mg·min/m^3	Concentration, mg/m^3	Duration of Exposure, min	Mortality Fraction	Time to Death, h[b]
Monkey	40,000	296	135	6/6	28, 43, 149, 190(2),* 248
	25,085	214	117	6/6	43, 47 67, 148, 235, 307
	20,800	219	95	4/6	42, 65, 238, 286
	16,720	209	80	3/6	192, 278, 350
	12,555	279	46	0/6	--
	5,040	297	20	0/6	--
Dog	16,720	209	110	6/6	10, 16, 17, 35(3)
	12,555	279	45	4/6	18, 20, 42, 116
	9,060	206	44	5/6	63, 86, 278, 136, 356
	5,940	297	20	1/6	305
	2,960	212	14	0/6	--
Goat	41,600	210	198	6/6	4, 16(2), 72, 77, 113
	30,000	227	132	6/6	22(2), 71, 95, 240, 552
	19,640	216	91	4/6	18, 90, 198, ?
	9,800	233	42	3/6	20(2), 239
	5,062	230	22	0/6	--
Swine	61,000	223	273	3/6	5.5, 20, 167
	41,600	210	198	2/6	4, 335
	10,000	227	132	2/6	47(2)
	19,640	216	91	1/6	42
	9,900	206	48	0/6	--
Rat	61,000	223	273	20/20	4, 8, 20(4), 47(5), 71, 95(2), 118(2), 124, 147(2), 163
	40,000	296	135	20/20	1(2), 47(2), 120(10), 190(4), 216(2)
	25,085	214	117	18/20	29, 110(12), 134, 158, 211(3)
	19,640	216	91	14/20	68(3), 140(3), 146, 148, 166(6)
	16,720	209	80	1/20	11
	12,555	279	45	1/20	21
	5,940	297	20	0/20	--
Guinea pig	16,720	209	80	17/20	11(6), 17, 35(7), 42, 64, 96
	12,555	279	45	19/20	19(14), 26(2), 528(2), 552
	5,940	297	20	11/20	16(8), 21(2), 40
	2,960	212	14	8/20	14, 16, 38(5), 70
	1,100	220	5	1/20	230
Rabbit	40,000	296	135	6/6	2.25(6)
	34,560	300	115	6/6	2(5), 24
	29,140	107	95	6/6	2(4), 2.5, 24
	20,900	279	75	6/6	1.5, 2, 24(3), 48
	11,070	246	45	4/6	1.2, 2, 24, 48
	8,050	268	30	4/6	24(2), 48, 72
	4,290	286	15	5/6	24, 72(2), 216, 240

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[a] Data from McNamara et al.[4]

[b] Numbers in parentheses are numbers of deaths at stated time, if other than 1.

TABLE 4-32

Mortality in Animals Exposed for 20 Consecutive Days at at Dosages Below LCt_{50}[a]

Species	Mortality after Single Exposure, %	Observed Mortality Fraction
Group 1--Average daily exposure, 11,609 mg·min/m^3:		
Monkey	3-13	5/8
Dog	1-6	1/8
Guinea pig	1.2-6	6/20
Group 2--Average daily exposure, 17,302 mg·min/m^3		
Monkey	16-46	8/8
Dog	8-28	2/8
Guinea pig	8-26	18/20

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[a] Data from Owens, *et al.*[5]

to the exposure. At the middle exposure, all superficial signs of exposure disappeared in 24 h. Two monkeys examined at 12 h showed bronchorrhea, focal pulmonary edema, and pulmonary congestion. At 24 h, edema and congestion were more pronounced. One monkey had membranous tracheitis and focal areas of hemorrhage into the parenchyma of the lung. At 72 h, edema and congestion had largely cleared. At 7 d, one animal appeared healthy, and the other had areas of emphysema and atelectasis. After 30 d, one monkey showed emphysema and atelectasis, and the other had extensive, early pneumonia. At the high exposure, eight monkeys died within 24 h. One animal killed for examination at 24 h showed early bronchopnemonia, pulmonary edema, emphysema, ulceration of the tracheobronchial tree, and some visceral congestion. The last monkey died at 29 d with the same array of lesions. No abnormalities in serologic or hematologic values were found in any of these animals. In summary, the most important health problem resulting from exposure to DM was pulmonary edema. This seemed to reach a peak at 24 h. If the animals survived beyond that time, they had little residual damage.

Striker *et al.*[11] considered that, at high exposures, animals may have held their breath. They may also have diluted or washed out some of the agent by coughing, gagging, etc. Lower exposures were studied in which seven groups of five monkeys were exposed to DM generated from an M6A1 grenade as follows:

Concentration, mg/m^3	Duration of Exposure, min	Ct, mg·min/m^3
291	2	582
291	10	2,910
272	20	5,440
330	40	13,200
99	2	198
108	12	1,296
77	60	4,620

At 582 mg·min/m^3, the first three pairs of monkeys showed only slight pulmonary congestion; at 1 wk and 30 d, congestion and edema were greater. At 2,910 mg·min/m^3, focal pulmonary edema and bronchorrhea were present at 12 h; the edema cleared by 24 h, but bronchorrhea and bronchitis persisted to 30 d. At 5,440 and 13,200 mg·min/m^3, lesions were similar, but increasingly severe. When the concentration of DM was reduced to about 100 mg/m^3, the external signs of intoxication were mild. At 198 mg·min/m^3, edema, congestion, and bronchorrhea were evident when the lungs were examined; at 72 h, the edema and congestion had cleared, and the bronchorrhea was likewise clear by 1 wk; these lesions persisted at 30 d. At 1,296 mg·min/m^3, the lesions were somewhat intensified, persisting up to 30 d. Surprisingly, in animals exposed to 4,620 mg·min/m^3, signs of labored breathing and edema were marked, but edema, congestion, and bronchorrhea, present up to 72 h, were not found at autopsy at 7 and 30 d. These results seem to bear out the original hypothesis of the authors that exposures to DM at fairly low Ct but for long periods produce relatively more severe effects than short exposures at high Ct.

Roberts et al.[8] examined the effects of DM on the gastrointestinal tract as a possible factor in poisoning. Using both intravenous and oral lethal doses in dogs, they monitored central venous pressure, right ventricular pressure, cortical electric activity, alveolar CO_2, respiratory rate, heart rate, electrocardiogram, and gastric activity. Both amplitude and rate of gastric activity were greatly increased by the introduction of DM, lasting for 15-20 min before decreasing to normal. Pretreatment with trimethobenzamide HCl, which prevents vomiting in dogs given peripherally or centrally acting emetics, did not counteract the DM-induced gastric activity, but chlorpromazine was effective, probably because of its atropine-like action. Blocking the nervous supply to the stomach did not cancel the DM effect. The authors concluded that DM affects the stomach directly and that the primary cause of death is its effect on the lungs, as seen in the experiments of Striker et al.

Owens et al.[5] tested the effects of DM suspensions in corn oil on rabbit eyes. DM was instilled in the eye in doses of 0.1, 0.2, 0.5, 1.0, and 5.0 mg. At 0.1 mg, there was no effect. Mild conjunctivitis was seen at 0.2 mg; at 0.5 mg, mild blepharitis was also seen. Corneal opacity persisting over the 14-d observation period resulted from 1.0 and 5.0 mg. Corn oil suspensions of DM (100 mg/ml) were applied to the clipped backs of rabbits in groups of six in doses of 1, 10, 50, 75, and 100 mg. At 10 mg and higher, necrosis of the skin was observed.

Rothberg[9] tested DM for skin sensitization in guinea pigs; the findings were negative.

TOXICITY IN HUMANS

Ballantyne[1] described the effects of human inhalation exposures to DM as beginning with acute pain in the nose and sinuses. Pain in the throat and chest follow, with sneezing and violent coughing. Next there is eye pain, lacrimation, blepharospasm, rhinorrhea, salivation, nausea, and vomiting. Recovery is usually complete 1-2 h after exposure. The onset of symptoms is, however, delayed for several minutes, in contrast with the onset of symptoms after exposure to CS and CN, which have very short latent periods--this permits the absorption of much more DM before a warning is perceived.

Owens et al.[51] estimated that threshold doses of DM for irritation of the throat, irritation of the lower respiratory tract, and initiation of the cough reflex are 0.38, 0.5, and 0.75 mg/m^3, respectively.

Incapacitating or riot-control agents are used to put persons out of action. DM is less effective in this regard than CS and CN. McNamara et al.[4] and Owens et al.[5] conjectured that there may be greater differences among persons in their susceptibility to DM than in their susceptibility to other agents. Although Owens et al. claimed that thousands of people were exposed to DM (no details given), few reports involved controlled tests.

Recent tests of the ICt_{50} for humans were probably more reliable than tests soon after World War I, but information is limited. McNamara et al.[4] summarized the results of two tests[3,6] by saying that "human exposures . . . indicated that men could tolerate concentrations of 22 to 92 mg/m^3 for 1 min or more"; "a concentration range of 22 to 220 mg/m^3 would appear to be intolerable to 50% of a population for 1 min"; and "these values are applicable to experimental situations."

In 1959, toxicity data for mice, guinea pigs, and dogs were combined to give an estimated LCt_{50} for humans of 14,000 $mg \cdot min/m^3$ for a single exposure. More recent estimates for three means of dispersion,[4] based on seven animal species combined, are as follows:

Form of Dispersion	ICt_{50}, $mg \cdot min/m^3$	ICt_{50}, $mg \cdot min/m^3$	Safety Factor
Laboratory dispersions	11,000	22 or 220	50-500
M6A1 grenade	44,000	22 or 220	200-2,000
Commercial grenade	35,000	22 or 220	160-1,600

McNamara *et al.*[4] reported the accidental death of a man exposed for an uncertain time (5-30 min) to DM at a concentration estimated at 1,130-2,260 mg/m^3.

No investigations of long-term effects of DM on humans or animals appear to have been undertaken.

EFFECTS ON HUMAN SUBJECTS AT EDGEWOOD

Sixty-seven human subjects underwent experimental exposure to DM: 29 in 1958 and 38 in 1966-1968, of whom exposure data are available on 23 and 31, respectively. Subjects were exposed in an aerosol chamber; they wore masks when they entered, and the masks were often removed after some intervals in the chamber.

The subjects tested in 1958 underwent one to five exposures on 1 or 2 d. Ct's, available for at least one exposure of 14 of the 1958 subjects, ranged from 7.1 to 100 $mg \cdot min/m^3$; exposure times, available for eight subjects, ranged from 1 min to 4 min 28 s. Subjects tested in 1966-1968 underwent only one exposure each; Ct's and exposure times, available for 31 subjects, were 7.1-236 $mg \cdot min/m^3$ and 45 s to 10 min, respectively.

Some subjects were transiently sickened by their exposures. Effects lasted up to several hours after removal from the agent. Predominant symptoms were related to respiratory tract irritation: burning sensations of the respiratory passages, choking sensations, dysphonia, dyspnea, coughing, and sneezing. Nausea was common. Other, less frequent effects were retching, anorexia, headache, dizziness, lacrimation, salivation, and urinary frequency. Objective signs of DM exposure were infrequent and included rhinorrhea, wheezing, conjunctivitis, and diaphoresis.

Laboratory results from 7 d after exposure are available for 31 of the 1966-1968 subjects. No abnormalities in routine clinical blood differential counts and urine analyses not seen before exposure were seen 7 d after.

Although DM has greater acute toxicity to the respiratory tract than CS and CN, Edgewood subjects appeared to recover shortly after exposure. Given the available information on DM and the short low-dose exposures, it is impossible to predict whether Edgewood subjects exposed to DM will suffer any long-term effects of the exposure.

SUMMARY

DM is a moderately toxic riot-control agent that produces symptoms of slightly delayed onset and a relatively long recovery period. Because they cause symptoms of more rapid onset and recovery and are less toxic, CS and CN may be said to be more effective riot-control agents and to make DM obsolete as a riot-control agent.

Castro[2] found DM and CS to be active inhibitors of cholinesterase and suggested that this characteristic might explain their lacrimatory effect. Roberts *et al.*[8] demonstrated a direct effect of DM on gastric activity and, like Striker *et al.*,[11,12] found solid evidence that its lethal effects are of respiratory origin.

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BROMBENZYL CYANIDE

CHARACTERISTICS

Brombenzyl cyanide (CA)--also called α-bromobenzyl cyanide, α-bromotolunitrile, bromobenzylnitrile, and α-bromophenylacetonitrile--was the most powerful lacrimator used in World War I (see Table 4-1). It was introduced by the French army and adopted as the standard U.S. lacrimator in 1918. It was temporarily abandoned because of its reactions with metal and instability in storage.[3,10] Interest in it revived when a need arose for a chemical that was more persistent than CS and CN in enclosed areas, such as rooms and earthen tunnels.[1] In this connection, vapors of CA from ground contamination are lacrimatory for 15-30 d.[6]

CA is a yellow-white solid, with a molecular weight of 195.97, a melting point of 25°C, a boiling point of 225°C[10] and the odor of sour fruit. Sim described its activity as immediate in onset and causing eye and respiratory irritation. Rapid recovery (2-10 min) from its effects is produced by moving to fresh air. It is low in skin irritancy.[93]

Witten[10] reported that tolerance to CA develops more easily than tolerance to CS. In an atmosphere containing CA at 0.44 mg/m^3, subjects lacrimate freely in 30 s. While still in the CA atmosphere, they stop lacrimating in 7-15 min. Exposure at a Ct of 5 mg·min/m^3 is incapacitating,[7] but Oberst *et al.* reported that men exposed in a chamber tolerated 30 mg/m^3 for 10 min.[5]

A comparison of some other characteristics of CA, CN, and chloropicrin (PS) follows:[6]

Substance	Volatility at 20°C, mg/m^3	Threshold Lacrimatory Concentration, mg/m^3
CA	130	0.15
CN	106	10.30
PS	165,000	2.00

TOXICITY IN ANIMALS

Oberst *et al.* [3] questioned the reliability of LCt$_{50}$s for CA in early reports. They then tested eight species of animals at "high" concentrations (105-168 mg/m^3 for 12-120 min) in an exposure chamber, using CA of 90% purity. Table 4-33 shows the concentrations, exposure times, mortality fractions, and times to death, and Table 4-34 summarizes the LCt$_{50}$s.

TABLE 4-33

Mortality of Animals Exposed to CA Vapor at High Concentrations (10-day Observation)[a]

Species	Ct, mg·min/m^3	Concentration, mg/m^3	Duration of Exposure, min	Mortality Fraction	Time to Death, h[b]
Mouse	6,120	136	45	1/10	168
	8,400	140	60	5/10	144(5)
	10,500	140	75	10/10	16.5, 40.5, 64.9(3), 88.5(3), 136.5, 216
Rat	5,850	130	45	0/8	--
	6,120	136	45	0/6	--
	8,040	134	60	0/7	--
	8,400	140	60	0/6	--
	10,500	140	75	0/6	--
	11,880	132	90	1/6	96
	15,960	133	120	4/7	24(2) 120(2)
	17,850	119	150	2/8	22, 120
	21,420	119	180	5/8	17(5)
Guinea pig	5,850	130	45	1/8	120
	6,120	136	45	1/6	114.5
	8,040	134	60	0/7	--
	8,400	140	60	2/5	151(2)
	10,500	140	75	4/6	64, 136(2), 161.5
	11,880	132	90	5/6	1, 40, 88(2), 94
	15,960	133	120	6/7	41.5, 137.5(5)
	17,850	119	150	6/8	18.8, 118.8(4), 138.8
	21,420	119	180	8/8	3(2), 15.8(3), 111.8(3)
Rabbit	5,850	130	45	1/8	368
	8,040	134	60	4/7	20.6, 53.3, 140.9(2)
	10,500	140	75	4/6	140.9(2)
	10,500	140	75	4/6	20, 22.7, 88, 336
	11,880	132	90	6/6	16, 40, 64, 88(3)
	15,960	133	120	7/7	1, 2, 17.5, 41.5(2), 45, 65.5
Monkey	8,400	140	60	0/3	--
	12,690	141	90	2/7	72, 144
	17,040	142	120	4/8	24, 72, 77, 78
	22,050	147	150	5/6	36(2), 71, 144, 312
Dog	8,400	140	60	0/2	--
	10,200	136	75	1/4	48
	13,320	148	90	6/8	23(2), 25, 36, 46, 120
	17,340	145	120	5/6	28, 36, 47(2), 168
Pig	1,548	129	12	0/6	--
	1,932	161	12	1/6	16.7
	2,340	117	20	0/4	--
	3,150	105	30	1/6	21.9
	4,680	156	30	2/6	16.5(2)
	5,640	141	40	3/6	5.5(2), 20.9
	6,120	136	45	3/3	48, 72(2)
	10,500	140	75	3/3	1.3, 16(2)
Goat	3,990	133	30	0/6	--
	6,160	154	40	0/6	--
	6,840	152	45	2/6	18.5, 120
	6,943	131	53	0/6	--
	10,080	168	60	5/6	16(2), 18.5, 23.5, 96

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[a] Data from Oberst *et al.*[3]

[b] Numbers in parentheses are numbers of deaths at stated time, if other than 1.

All species except rats and guinea pigs responded to CA vapor rapidly. All had lacrimation and salivation, with various kinds of eye-rubbing, blinking, licking, and vomiting. Goats were the least responsive. Dogs, rabbits, and monkeys seemed most susceptible to eye damage. Conjunctivitis, blepharospasm, and corneal ulcers were common. The rhesus monkey appeared most susceptible to cutaneous irritation; dogs and pigs seemed non-susceptible in this regard. Respiratory damage was common and appeared to be the cause of death in animals that did not survive. The data from all species were combined for an LCt_{50} value of 11,095 mg·min/m^3. This was considered a tentative value for humans.

Oberst et al.[4] also examined the toxicity of CA at lower concentrations. In this series of tests (at 8-29 mg/m^3 for up to 168 h), five species were used. Table 4-35 shows the mortality fractions; relatively few animals died. Dogs were most likely to die, possibly because, being mongrels, they were in poor health to begin with.

Signs of CA irritation were similar to those seen at high concentrations, but less severe. Corneal damage seen in monkeys and rabbits early during exposure disappeared while the animals were still in the test chambers. Survivors showed no apparent after-effects. No estimates of ICt_{50}s or LCt_{50}s were made from these data.

LCt_{50}s for CA disseminated from the M6 oil candle were obtained by Ballard et al.[1] These experiments were performed in generally the same way as those done by Oberst et al., but this method of agent dispersion produced both vapor and aerosol in widely varying proportions; the concentration of CA also varied widely (255-1,707 mg/m^3). The LCt_{50}s for the four species used are therefore not reliable. A comparison of these values with those reported by Oberst et al.[3] suggests that combustion products from the candle may have been partly responsible for the deaths.

	LCt_{50}s, mg·min/m^3	
Species	Ballard et al.	Oberst et al.
Monkey	10,831	16,287
Dog	4,675	12,037
Rat	11,740	18,859
Guinea pig	4,785	10,214

Bezahler et al.[2] performed pathologic examinations on 207 animals of various species that died during or after the previously described exposures. Controls were not available. Lesions were not distinguishable from those produced by common laboratory animal diseases, and an arbitrary decision was made that CA was responsible for death if it occurred within 6 h of exposure. Laryngeal edema was commonly seen, and respiratory lesions were generally the cause of death.

A second arbitrary group was established for animals that died 6-24 h after exposure. Tracheobronchitis and bronchopneumonia appeared to be the causes of death, except that in half the rats and two-thirds of the guinea pigs the lesions were insufficient to explain their deaths. After 24 h, nearly all the animals that eventually died had severe respiratory lesions. In the absence of controls, the investigators were reluctant to say that CA caused the lesions found in survivors.

Tests of guinea pigs for skin sensitization potential with CA yield negative results; CN and CS were positive.[8]

TOXICITY IN HUMANS

Although the human LCt_{50} was arbitrarily set by combining values for eight animal species (Table 4-34), neither ICt_{50} for humans nor long-term effects in humans or animals have been reported.

EFFECTS ON HUMAN SUBJECTS AT EDGEWOOD

In 1966, 13 human subjects underwent experimental exposures to CA at Edgewood. The subjects wore masks when they entered an aerosol chamber and removed the masks after equilibration of the chamber CA concentration.

Each subject had only one CA exposure. The exposure Ct's ranged from 0.9 to 204 $mg \cdot min/m^3$. Exposure times, available for 11 subjects, ranged from 50 s to 10 min.

Effects of CA exposure were transient. Ocular irritation, often accompanied by conjunctivitis, was predominant. Upper respiratory tract irritation with rhinorrhea also occurred.

Routine blood differential counts and urinalyses analyses obtained 7 d after CA exposure are available on 12 subjects. One subject had minimal leukocytosis (WBC, 12,800) that was not seen in his pre-exposure laboratory analyses.

Exposure was for a short term and at a low dose, however, given the absence of data on chronic effects it is not possible to estimate long term health effects of the CA exposure.

SUMMARY

CA, the most powerful lacrimator used in World War I, was discarded in favor of CN because of storage stability problems resulting from its reactivity with steel and iron containers. A resurgence of interest during the 1960s led to its use to clear persons out of tunnels in Vietnam. The LCt_{50} for humans has been estimated at 11,095 $mg \cdot min/m^3$ on the basis of animal data. CA is not a skin irritant or skin sensitizer in animals.

TABLE 4-34

CA Vapor LCt_{50}s for Eight Animal Species[a]

Species	LCt_{50},[b] mg·min/m^3	95% Confidence Limits
Mouse	7,968	7,100 - 8,942
Rat	18,859	15,151 -23,474
Guinea pig	10,214	8,580 -12,160
Rabbit	8,021	6,714 - 9,582
Monkey	16,287	13,132 -20,201
Dog	12,037	9,161 -15,815
Pig	4,852	3,580 - 6,576
Goat	8,401	7,043 -10,020
All species	11,095	9,661 -12,741

Monkeys--Monkeys usually were blinking and rubbing their eyes within 1 min. They kept their eyes partially closed most of the time. As early as 26 min, most monkeys appeared to be distressed; they were salivating and breathing through their mouths. In 80 min, some were gasping and making choking sounds.

Dogs--Immediately after the dogs were placed in the chamber, they began to blink and lick their mouths. Occasionally, choking was observed at 30 s. They were salivating within 2.5 min. Rhinorrhea, partially closed eyes, and vigorous scratching at the window to escape usually were noted in 5 min. By 28 min, salivation was profuse, and headshaking, retching, and corneal opacity were present. Vomiting and phonation occurred in 1 h, dyspnea and coughing in 2 h.

Pigs--Usually, pigs were active, salivating, blinking, and shaking their heads in 30 s. Difficult breathing (mouth breathing) was observed in 15 min. At approximately 18 min, they fought, squealed, and either closed or partially closed their eyes. Occasionally, vomiting was observed in 30 min.

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[a] Data from Oberst *et al.*[3]

[b] Concentration, 105-168 mg/m^3.

TABLE 4-35

Mortality of Animals Exposed to CA at Low Concentrations[a]

Run No.	Avg. Concentration, mg/m^3	Duration of Exposure, h	Avg. Ct,[b] $mg \cdot min/m^3$	Mortality Fraction: Mouse	Rat	Guinea Pig	Rabbit	Monkey	Dog	Guinea Pig	Goat
27	10.1 (6.9 - 13.1)	12	7,272	0/10	0/6	0/6	0/6	2/6	5/6	0/6	0/6
36	8.1 (7.1 - 9.5)	8	3,888	--	--	--	--	--	0/6	--	--
36	8.5 (7.7 - 10.6)	12	6,120	--	--	--	--	--	2/3	--	--
46	8.4 (7.1 - 10.6)	18	9,072	0/9	0/6	0/6	1/6	0/6	1/3	0/6	1/4
41	11.7 (9.7 - 13.9)	9	6,318	--	--	--	--	--	2/6	--	--
41	11.3 (9.7 - 13.9)	12	8,136	--	--	--	--	--	0/3	--	--
43	15.4(12.9 - 18.0)	24	22,230	4/10	0/6	0/60	0/6	0/6	5/6	0/6	0/6
44	12.7 (7.7 - 20.6)	24[c]	18,288	--	--	--	--	--	1/1	--	--
44	12.6 (7.7 - 18.2)	34	25,826	--	--	--	--	--	2/6	--	--
44	14.0 (7.7 - 20.6)	40	33,686	0/10	0/6	0/6	1/6	0/6	--	0/6	0/6
45	12.7 (9.0 - 18.9)	44.5	40,320	--	--	--	--	--	2/2[d]	--	--
45	13.6 (9.0 - 18.9)	54.5	45,690	--	--	--	--	--	1/1[d]	--	--
45	17.3 (9.0 - 18.9)	90	83,331	--	--	--	--	--	1/1[d]	--	--
45	11.0 (9.0 - 18.9)	100	86,538	--	--	--	--	--	1/1[d]	--	--
45[e]	12.3 (4.9 - 18.9)	168	124,108	0/10	0/6	1/6	1/6	0/6	0/1	0/6	0/6

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[a] Data from Oberst *et al.*[4]

[b] Cumulated each time concentration of a sample was determined.

[c] Estimated.

[d] Dogs died during exposure.

[e] Except for dogs, two animals of each species were necropsied immediately after exposure, two animals after 1 wk, and two animals after 4 wk (one rabbit and one guinea pig at 4 wk).

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CHLOROPICRIN

CHARACTERISTICS

Chloropicrin (PS)--trichloronitromethane, CCl_3NO_2, Green Cross, Klop--is a colorless, volatile, slightly oily liquid with an intense odor (see Table 4-1). It is very slightly soluble in water, soluble in ether, and miscible with benzene, absolute alcohol, and carbon disulfide.[10,11] It boils at about 112°C.

Used extensively as a harassing agent in World War I, PS is a lacrimator and a lung irritant with a tendency to cause nausea and vomiting. In acute toxicity, it is intermediate between chlorine and phosgene;[10] unlike phosgene, it has no latent period. During Oorld War I, it was known in Germany as Green Cross, because artilOery shells were so marked,[2] and Klop, because it was made at the Klopper Werke at Breloh in the Lüneberg Heath. Büscher[2] described PS as a fairly volatile liquid, resisting decomposition by water; it was often combined in a ratio of 35:65 with diphosgene as a charge for shells. Sim[9] said that it was no longer of interest as a military chemical.

PS is used as an insecticide and sterilizing agent, mainly for cereal grain in storage or in ships' holds. As a soil fumigant, it is used to control nematodes, microorganisms, and weed seeds. Treated soil must be left unplanted for about 2 wk, because PS damages seeds. It is also a warning agent in fumigants, such as methyl bromide, because of its strong odor.[15,16] At 1 ppm in air, it causes lacrimation and smarting pain.[3]

TOXICITY IN ANIMALS

Toxicity data on PS are scanty. Short-term studies have been extremely limited, and long-term studies are lacking.[15,16]

The 1980 Registry of Toxic Effects of Chemical Substances[6] listed the following animal-toxicity data:

- Oral: rat LD_{50}, 250 mg/kg.
- Intraperitoneal: mouse LD_{50}, 25 mg/kg.
- Inhalation: mouse LC_{50}, 1,600 mg/m^3 for 10 min.
- Inhalation: cat LC_{Lo}, 800 mg/m^3 for 20 min.
- Inhalation: rabbit LC_{Lo}, 800 mg/m^3 for 20 min.
- Inhalation: guinea pig LC_{Lo}, 800 mg/m^3 for 20 min.

Silver et al.[8] reported a median lethal concentration of 1,500 mg/m^3, by inhalation, for 10-min exposure of mice. Because the 11 separate runs of 20 mice varied widely in their LC values 10 d after exposure, Silver et al. noted that physiologic variations were caused by differences in age, nutrition, etc.

The United States Testing Co. performed tests to measure the toxicity of PS administered by inhalation, by mouth, and cutaneously.[4] Conditions and results were as follows:

- Inhalation toxicity: The LC_{50} was the concentration that would kill 50% of the population within 14 d when administered by continuous inhalation (over 90% of particles under 10 um) for 1 h. Five male and five female Sherman adult rats were used at each dose. LC_{50}: 25.5 ppm.

- Oral toxicity: Animals used were as above. The LD_{50}0was the dose that would kill 50% of the animals within 14 d when given orally by intubation. LD_{50}: 37.5 mg/kg.

- Skin absorption: The LD_{50} was the dose that would kill 50% of the test animals within 14 d after continuous contact with the skin of rabbits for 24 h. No data on numbers of animals were given. LD_{50}: 100.0 mg/kg.

- Skin corrosion: The dose sought was the dose that would cause visible destruction or irreversible alteration in skin tissue, such as ulceration or necrosis, after 4 h of contact with the skin of rabbits. Six albino rabbits with clipped backs were used for each dose. Corrosive dose: 0.5 ml (the only dose listed).

TOXICITY IN HUMANS

PS may enter the body through the skin, respiratory tract, or gastrointestinal tract. It has a pungent odor and its effects are evident several hours after exposure. The acute effects of PS include burning eye discomfort, lacrimation, headache, photophobia, burning sensation in the nose and throat, coughing, nausea and vomiting with colicky abdominal pain, diarrhea, and occasionally pulmonary edema.[5] The concentration necessary to cause fatal pulmonary edema is 2.4 g/m^3, and methemoglobinemia has been associated with PS intoxication/[15].

Although PS has lost status as a chemical-warfare agent, its toxic potential makes it an industrial and agricultural hazard. The Occupational Health Guideline[14] described it as a severe irritant of the eyes, skin, and respiratory tract. Exposure of humans at 119 ppm for 30 min results in pulmonary edema and death. The Guideline gave an extreme tolerance time of 1 min at 15 ppm, but added that a few seconds at 4 ppm is disabling. Concentrations of 0.3-0.37 ppm result in painful eye irritation in 3-30 s. The permissible concentration in the workplace is 0.1 ppm (0.7 mg/m^3), averaged over an 8-h day.[14] The Guideline stated that, even though PS is not a skin sensitizer, persons exposed to it once show reduced tolerance to other toxic effects.

A Center for Disease Control publication[12] described the effects of a PS-containing mixture of methyl bromide used to fumigate a 15-acre field near suburban housing. Although the field was covered with plastic sheeting after fumigation, 35 (26%) of 133 nearby residents developed eye irritation, nausea, coughing, vomiting, or other typical symptoms of exposure to PS a few hours later.

A similar public-health problem was reported from Japan by Okada *et al.*[7] PS was used as a fumigant for leaf tobacco. Large numbers of farm workers reported symptoms. Of 760 workers and nearby residents surveyed, five required medical attention and 72% reported one or more symptoms. The five workers, evaluated more extensively, experienced lacrimation, vertigo, headache, fatigue, and orthostatic hypotension for several hours after exposure to PS. The other persons surveyed had lacrimation, headache, coughing, nausea, and anorexia for 1-3 d after exposure; and 30 had symptoms for over 1 mo after exposure. Age and symptoms were positively correlated.

MUTAGENICITY

Like many other irritants, PS has not been tested thoroughly for mutagenicity. However, in one early study, Auerbach[1] tested its capacity to induce sex-linked recessive lethal mutations in the fruit fly, *Drosophila melanogaster*. In all broods of flies derived from PS-treated males, the mutation frequencies were consistent with those of untreated laboratory stocks. A total of 4,454 chromosomes were tested, and the mutation frequency was 0.2%. In contrast, flies treated with mustard gas had a frequency of 5.2%.

The sex-linked recessive-lethal test in *Drosophila* continues to be highly regarded in modern genetic toxicology. In current mutagenicity testing, however, it is generally recommended that tests be conducted in more than one experimental organism and that more than one genetic end point be studied.

Because we have found no evidence in the scientific literature that PS has been studied in other mutagenicity tests, some uncertainty remains in categorizing it as nonmutagenic.

CARCINOGENICITY

The National Cancer Institute[13] studied the carcinogenicity of PS. Osborne Mendel rats and B6C3F1 mice were given PS in corn oil by gavage. Rats of both sexes were initially given 46 and 23 mg/kg per day, but the dosage for males was increased to 56 and 28 mg/kg per day at week 5, because males appeared to tolerate the agent better than females. Some early deaths caused periodic suspensions of the dosing, so the overall pattern was intermittent. Dosing was terminated at 78 wk, but observation continued for an additional 32 wk. Mice were given 50 and 25 mg/kg per day. At week 14, the dosage was increased to 70 and 35 mg/kg per day for a total of 78 wk. Controls were treated with corn oil. Animals were necropsied, and slides prepared from 29 different tissues. Survival curves for rats and mice are shown in Figures 4-4 and 4-5.

No statistically significant differences in tumor incidence were found in rats or mice. It was concluded that, because of high early mortality, too few rats lived long enough to develop late-appearing tumors.

EFFECTS ON HUMAN SUBJECTS AT EDGEWOOD

From 1955 to 1971, 136 subjects underwent one or two experimental exposures to PS in equipment tests. The subjects remained in a test chamber for up to 4 h with gas masks in place and sometimes simulated battlefield functions.

Exposure records are available on 46 subjects exposed to PS vapor in 1955 and 12 subjects exposed in 1967. No dosages are listed. Data are not available on 17 subjects exposed to PS in 1955, 23 in 1956, nine in 1960, 11 in 1965, nine in 1967, three in 1969, and six in 1971.

Apparently, there was minimal gas-mask leakage during the experiments involving the 58 subjects on whom there are records, and the PS had no acute effects on these subjects. No laboratory analyses are recorded.

Some experimental information on PS indicates a possibility of skin absorption. During the Edgewood experiments, effective masks seemed to prevent acute inhalation toxicity, but subjects exposed to PS vapor for 4 h might have absorbed small amounts through their skin.

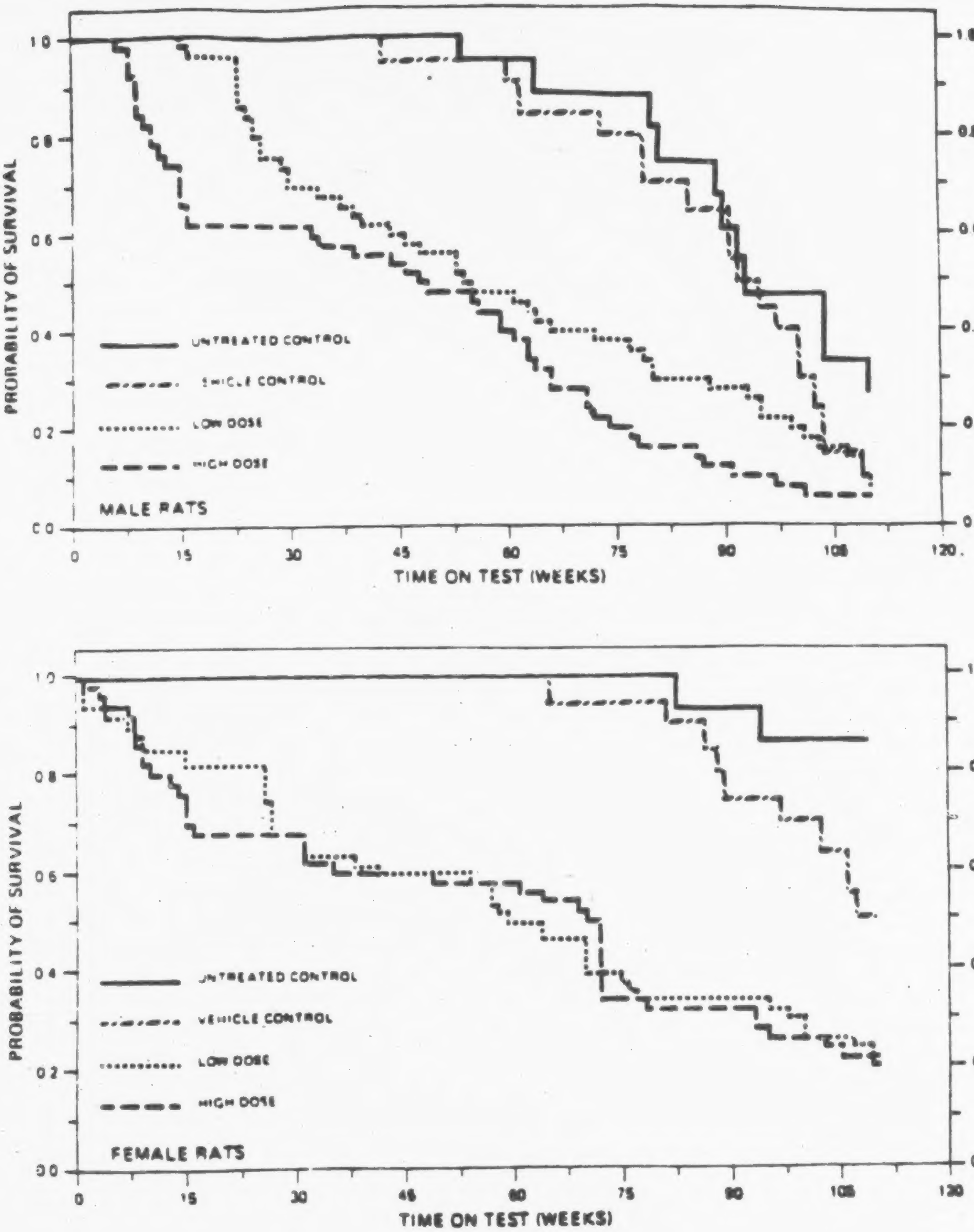

FIGURE 4-4 Survival of rats exposed to PS. Data from U.S. National Cancer Institute.[13]

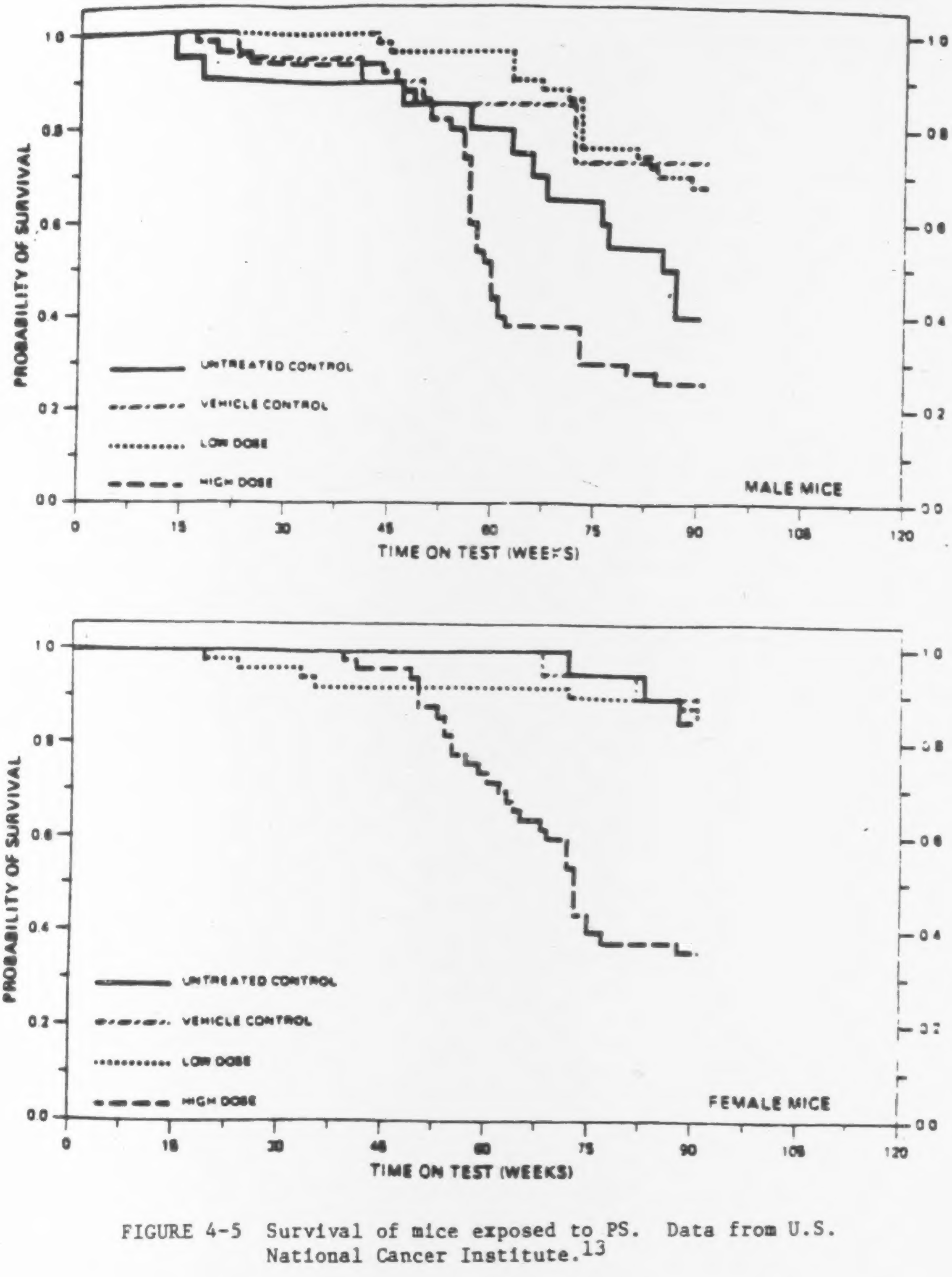

FIGURE 4-5 Survival of mice exposed to PS. Data from U.S. National Cancer Institute.[13]

PS is acutely quite toxic to humans, but little is known of its long-term health effects.

DISCUSSION

Despite widespread use as an insecticide, nematocide, and intermediate in chemical processes, the toxic potential of PS has not been fully evaluated. Absence of lethal effects may well be explained by its powerful odor, which alerts workers to its presence in contaminated areas. Animal-toxicity data suffer from variations in the methods used. One long-term study of carcinogenicity in rats was unsatisfactory because of high early mortality.

SUMMARY

PS is acutely toxic, with a variety of effects on animals. PS has not been tested thoroughly for mutagenicity. A test for sex-linked recessive lethal mutations in Drosophila was negative. However, because we have found no evidence that PS has been studied in other mutagenicity tests, uncertainty remains in categorizing it as nonmutagenic. A single carcinogenicity test in rats and mice yielded no evidence of carcinogenicity, but the test was flawed by the occurrence of deaths among experimental animals.

Because of its widespread use in agriculture, transportation, and industrial chemistry, further studies of its chronic toxicity and long-term effects are needed.

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NONANOYL MORPHOLIDE

CHARACTERISTICS

Nonanoyl morpholide--pelargonic morpholide or EA 1778--is a fatty acid amide with a molecular weight of 227 and a boiling point of 120-130°C at 0.5 mm Hg (see Table 4-1). Classed as a pungent, because of its pungent odor, it is chemically related to alkaloids found in black pepper.[1,3,4] Its synthesis has been described by Rice _et_ _al_.[3]

TOXICITY IN ANIMALS

The available data on this compound are sparse. Two reports were obtained, one on animal, the other on human exposures.

Punte _et_ _al_.[1] compared the toxicities of CN, DM, and nonanoyl morpholide in rabbits, rats, mice, and guinea pigs. Results are given below.

- Intravenous toxicity: The acute LD_{50} in rabbits is about 21 mg/kg, making it comparable with CN and somewhat less toxic than DM by this route (Table 4-36).

- Ocular toxicity: Instillation of 0.5 or 1.0 mg into the rabbit conjunctival sac produced mild to moderate conjunctivitis that lasted a week. If the eyes were washed out with water a few minutes after dosing, the conjunctivitis lasted 24 h.

- Inhalation toxicity: Aerosol exposure (mass median diameter, 1.8 μm) produced similar toxic signs in rats, mice, and guinea pigs. A brief period of hyperactivity was followed by salivation and lacrimation. Lethargy and labored breathing began after 5-15 min and lasted 1-2 h. Other toxic signs subsided within minutes of removal to fresh air. Table 4-37 presents data on inhalation toxicity, and Table 4-38 compares nonanoyl morpholide with CN and DM for lethal effects.

The animals that died or were sacrificed for autopsy examination after exposure to nonanoyl morpholide showed hemorrhagic edema of the lungs. Their intestines showed hemorrhages, dilation, and degeneration of the villi and loss of epithelium in some areas.

Results on animals were used to estimate a maximal safe inhalation Ct of 500 mg·min/m^3 for human exposures.

TABLE 4-36

Intravenous Toxicity of CN, DM, and Nonanoyl Morpholide in Rabbits[a]

Compound	No. Tested	LD_{50}, mg/kg
CN	20	20
DM	32	6
Nonanoyl morpholide	18	21

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[a] Data from Punte *et al.*[1]

TABLE 4-37

Computed Inhalation LCt_{50}s of CN, DM, and Nonanoyl Morpholide in Various Animal Species[a]

	Rats		Mice		Guinea Pigs	
Compound	No. Tested	LCt_{50},[b] mg·min m^3	No. Tested	LCt_{50},[b] mg·min/m^3	No. Tested	LCt_{50},[b] mg·min/m^3
CN	80	3.7	80	78.6	70	3.6
DM	24	3.7	42	22.4	56	7.9
Nonanoyl morpholide	60	58	60	130[c]	60	19.0

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[a] Data from Punte et al.[1]

[b] Multiply values by 1,000 to obtain LCt_{50}.

[c] Estimated by extension of dosage-mortality curve.

TABLE 4-38

Computed Inhalation LD_{50}s for CN, DM, and Nonanoyl Morpholide[a]

	LD_{50} mg/kg[b]		
Compound	Rats	Mice	Guinea Pigs
CN	14.1	58.8	1.1
DM	14.1	17.9	2.4
Nonanoyl morpholide	23.2	104	5.7

---

[a] Data from Punte *et al.*[1]

[b] Based on minute volume, retention, and body weight.

TOXICITY IN HUMANS

Using results on animals as a basis, Punte *et al.*[2] exposed healthy subjects to nonanoyl morpholide at several concentrations. Response times are shown in Figure 4-6. Table 4-39 compares ECt_{50}s of nonanoyl morpoholide, DM, and CN. The human responses to exposure sometimes provoked comments that compared nonanoyl morpholide with pepper. The symptoms after 3-min exposure at a Ct of 20-40 $mg \cdot min/m^3$ were lacrimation, coughing, and a burning sensation of the nose, throat, and eyes. Nausea sometimes followed, but only if the subject had recently eaten a large meal. Symptoms were relieved in 10-15 min by exposure to fresh air.[4]

EFFECTS ON HUMAN SUBJECTS AT EDGEWOOD

In 1958, 32 subjects underwent experimental exposure to nonanoyl morpholide in an aerosol chamber at Edgewood. At least some subjects wore masks during exposure. Exposure data are available on 30 subjects. Subjects had one to four exposures on 1 or 2 d. Exposure durations, available for 15 subjects, ranged from 10 s to 8 min. No Ct's are avilable.

The effects of nonanoyl morpholide exposure on the subjects were generally transient. The principal effects were due to respiratory tract irritation and included rhinorrhea, cough, substernal pain, and dyspnea. Nausea was also common, and vomiting occurred if the subject had eaten before the test. Headache sometimes occurred 1 h after exposure; in one subject, headache lasted for a week. Lacrimation, sometimes associated with conjunctivitis, occurred less frequently than other effects. No laboratory analyses are available.

DISCUSSION

Although they are not extensive, the data of Punte *et al.*[1,2] suggest that nonanoyl morpholide is less toxic than CN or DM. Nonanoyl morpholide appears more irritating than CN or DM, but may have less persistent effects. No definitive information is available on the possibility of long-term effects of exposure to nonanoyl morpholide. However, given the available information on nonanoyl morpholide and the short-term low-dose exposures of Edgewood subjects to it, the Committee believes that long-term health effects on the subjects are unlikely.

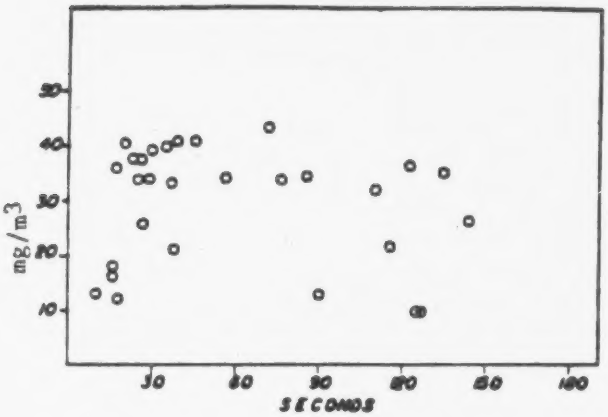

FIGURE 4-6 Response (tolerance time) of 26 subjects exposed to nonanoyl morpholide for 180 s. Reprinted with permission from Punte *et al*.[2]

TABLE 4-39

Estimated Airborne Ct Needed to Produce Response (Acute Irritation) in 50% of Subjects for Various Exposure Times to CN, DM, and Nonanoyl Morpholide[a]

Compound	1-Min ECt_{50}, mg·min/m^3	2-Min ECt_{50}, mg·min/m^3	3-Min ECt_{50}, mg·min/m^3
CN	213[b]	119	93
DM	[c]	38[b]	19
Nonanoyl morpholoide	39	29	21

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[a] Data from Punte *et al*.[2]

[b] Estimated on basis of limited data.

[c] Too few responses to make estimate.

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1-METHOXY-1,3,5-CYCLOHEPTATRIENE

CHARACTERISTICS

Riot-control agents--such as CS, CN, and CR--are solids. Edgewood scientists[9] felt that dissemination and decontamination would be simpler with a volatile liquid agent. Research led to the production and testing of 1-methoxy-1,3,5-cycloheptatriene (CHT, EA 4923), a liquid substance of high volatility (Table 4-40) with physiologic effects typical of riot-control agents (e.g., lacrimation, skin irritation, and mucous membrane irritation).

CHT, known as tropilidene in England,[2] is pale yellow, with a molecular weight of 92, a boiling point of 117°C at 749 mm Hg, and a freezing point of -79.5°C. It is unstable when exposed to air or light. Early Edgewood samples were difficult to produce in a pure state, ranging from 78 to 89%, with EA 4922 (a relatively inert isomer) forming most of the remainder.

CHT is less irritating than CS and CR to humans. It is roughly as toxic as CR and less toxic than CS by inhalation in animals, and it has the capacity to penetrate skin or rubber.[9]

TOXICITY IN ANIMALS

Brown *et al.*[2] evaluated the safe handling of CHT, using only four males and four females per test. They used 96% pure CHT. In gavage studies, the LD_{50} of CHT in rats and mice was 57 and 171 mg/kg, respectively. Clonic convulsions suggested CNS involvement as a mechanism of toxic effects. Undiluted CHT applied to clipped back skin of rats under an impermeable bandage for 24 h produced an LD_{50} of 442-884 mg/kg. When 1 ml of CHT was applied to a 2 x 2-in. patch that was then placed on the clipped back skin of rabbits under an impermeable cover, and this procedure was repeated daily for 3 d for 6 h at a time, severe damage was caused, with necrosis and ulceration. Application of 1 ml of CHT to the clipped, uncovered backs of two rabbits and 10 guinea pigs daily for 23 d, produced gross skin irritation, with spongiosis, acanthosis, and necrosis. Attempts to sensitize guinea pigs yielded negative results. Undiluted CHT applied to rabbit eyes caused severe conjunctivitis that cleared in 24 h; there was swelling of lids, but no corneal involvement.

Early studies at Edgewood were conducted on dogs.[9] Severe neurologic damage was observed in one animal dosed with degraded agent (a dark mixture with a nonvolatile component). This observation led to further studies, including the use of CHT stabilized with antioxidants. Percutaneous toxicity was tested by applying CHT to the backs of clipped dogs and covering the area with a Saran plastic sheet held in place with a cloth jacket. Jackets were removed after 24 h, and the dogs' skins were then decontaminated. Results (Table 4-41) showed that ataxia, tremors, and death can be produced by these procedures. Other tests involved topical applications of CHT at 0.5 and 1.0 ml/kg (86.7 mol% pure) on two sets of dogs; stabilized and nonstabilized material was used. Eight dogs (two per test) were tethered outdoors and exposed uncovered for 4 h at 75°F. A second group of eight dogs was similarly tested at 100°F. No dogs died, but one showed transitory mild tremors after application of the stabilized agent at 1.0 ml/kg. The percutaneous LD_{50} in dogs (occluded skin) was estimated at about 500 mg/kg. Results were similar for stabilized and nonstabilized CHT. Ocular instillation of 5 l (2 d), 10 l (1 d) and 50 l (4 d) of freshly distilled, about 90% pure agent caused mild erythema, inflammation, and iritis in rabbits, which cleared in 4-6 d. Other samples and degraded fractions given in single instillations of 10-50 l produced varied but increased eye injury, including permanent corneal opacity, and in some cases injury to skin.[9]

The combined effects of inhaled and topically applied CHT were tested on eight dogs on which stabilized and nonstabilized CHT was applied at 1.0 and 0.5 ml/kg.[4] The dogs were placed in an unventilated 441-ft^3 room whose atmosphere was saturated with CHT and exposed twice, once for 1.5 h and a second time for 6 h. Effects of

1.5-h exposures were long-lasting, but not lethal. Deaths occurred from the 6-h exposures, however (Table 4-42). The effects of inhaled CHT were studied in dogs by allowing CHT equivalent to 0.5 and 1.0 ml/kg to evaporate from absorbent cotton in a closed cubicle. No ill effects were seen after exposure for 1.5 h. The effectiveness of decontamination procedures with bleach or soap solution was tested (Table 4-43).

McNamara et al.[7] used several species of animals and several routes of administration. Various samples of CHT were used, both controlled (relatively pure and stabilized) and uncontrolled (containing degradation products). They stated that toxic signs associated with controlled and uncontrolled samples were often the same. The intravenous LD_{50}s of neat agent (uncontrolled samples) were 99, 88, and about 20 mg/kg in the mouse, rabbit, and dog, respectively. The percutaneous (covered-patch) LD_{50}s in rabbits varied from 480 to 1,000 mg/kg for different samples. The acute inhalation LCt_{50}s (controlled samples) were 184,000, 176,000, and 63,000 in the rat, guinea pig, and dog, respectively. Death usually occurred within 24 h of exposure to lethal doses. Neuromuscular weakness persisted for several months in some dogs after the cutaneous (covered) application of CHT at 500 mg/kg or intravenous administration at 10 mg/kg. Persistent weakness was seen in rabbits after cutaneous exposure. Some samples of CHT produced corneal lesions in rabbits. Necropsies showed no lesions after CHT was given intraperitoneally, intratracheally, ocularly, or cutaneously. Pulmonary lesions were found after intravenous administration to dogs and after inhalation by dogs, rats, and guinea pigs. In surviving dogs and guinea pigs, but not always in rats, the lesions were reversible.

Biskup et al.,[1] also at Edgewood, compared the parenteral toxicities of six agents, including CHT. LD_{50}s in the mouse, rat, and rabbit are shown in Table 4-44.

Toxicity studies were conducted at Stanford Research Institute (SRI) with CHT under contracts with Edgewood. The first part of the SRI work was concerned with the pulmonary and neurologic lesions caused by CHT in dogs and monkeys. Monkeys tolerated high concentrations of CHT for short times and low concentrations for periods up to 100 min without adverse, irreversible effects. Ct's of 985-105,100 $mg \cdot min/m^3$ were tested. No significant changes in blood pressure, heart rate, or respiration rate were observed. A comparison with CS indicated that CS can produce lung lesions at a Ct of 47,000 $mg \cdot min/m^3$ in dogs and 20,000 $mg \cdot min/m^3$ in monkeys. These are much lower than Ct's of CHT that produced no significant lesions. Neurologic lesions were produced by intravenous administration of CHT in dogs, but not in monkeys. This confirms the early results at Edgewood and suggests that dogs may be peculiarly susceptible to this type of treatment.[4] ^{14}C-labeled CHT was used to study distribution and excretion. No indication of retention in any organ was observed.[4] A summary of percutaneous toxicity of CHT in dogs with occluded dressings is in Table 4-45.[3] In a later study, the inhalation of CHT in goats was studied at SRI. Eighteen goats were tested: six were controls, six were given a low dose (Ct, 3,000 $mg \cdot min/m^3$), and six were given a high dose (30,000 $mg \cdot min/m^3$). Three of the six goats in the high-dose test died before the exposure ended, so useful data were not obtained. All exposures, however, lowered the white-cell count and the hematocrit. No change in blood-gas contents was seen. No significant changes in biochemical or pathologic factors were found.[10]

TOXICITY IN HUMANS

During the chemical synthetic work that led to the development of CHT, several men were exposed to its vapors. The resulting lacrimation and irritation of the mucous membranes passed quickly with no after-effects. The compound was therefore judged to be a potent irritant, but relatively harmless.[9] Because of these observations, human volunteers and animals were tested simultaneously at Edgewood.

Fourteen men were exposed to various concentrations of CHT to establish an ICt_{50}. Nine of these men withstood exposures of 28-64 $mg \cdot min/m^3$. These tests were terminated before an ICt_{50} could be established, because some animal tests suggested that the compound might be toxic at higher concentration.[7] Eventually, it was found that CHT had been degraded by exposure to air and light. Purified and stabilized agent was then prepared for further toxicity testing, but no further human tests were conducted at Edgewood. Accidental exposures reported by SRI involved ocular, cutaneous, and inhalation routes. Irritant effects developed at once and disappeared 15-30 min after removal of the agent. No after-effects were reported.[3]

MUTAGENICITY

CHT has not been tested thoroughly for mutagenicity. On the basis of unpublished studies conducted by Edgewood, it has been suggested that CHT is nonmutagenic in the *Salmonella* reverse-mutation test,[11] the micronucleus test in mice,[11] and a dominant-lethal test in rats.[6] Limitations in the available data base, however, make it impossible to reach clear conclusions regarding the genotoxicity of CHT.

The *Salmonella* reverse-mutation test reported for CHT does not seem to have been an adequate negative test. In strains TA1538 and TA100 with metabolic activation, the range of dosages was too limited, and there was no suitable positive control for strain TA1537. Moreover, the data on strain TA98 could suggest a positive response, in that there was a fourfold increase in numbers of revertants per plate. If this increase were reproducible, one could conclude that CHT is positive, rather than negative. The available information on the size of the experiments, their reproducibility, and the variability of the data is also too limited to support the general conclusion that CHT is nonmutagenic in bacteria.

It was reported that CHT was nonmutagenic in a test for dominant lethal mutations in rats.[11] Treatment was by inhalation at concentrations of 100 and 4,000 $\mu l/m^3$; treatment involved single 20-min exposures or five 20-min exposures on consecutive days. Not having reviewed the dominant-lethal test data, we can neither accept nor refute the claim that CHT was negative in this test. However, a negative result in dominant-lethal tests is not generally regarded as strong evidence of lack of genotoxicity.[8]

CHT was tested for its capacity to cause an increase in the frequency of micronuclei in polychromatic erythrocytes in mouse bone marrow. Micronucleus formation is an indicator of chromosomal damage, and CHT had no apparent effect in the test. It should be noted, however, that the procedures used may not have conformed to current standards for a sensitive micronucleus test. For example, Heddle *et al.*[5] recommended examining at least 500 polychromatic erythrocytes from each of at least eight animals for every dosage and time for which results were analyzed; they also recommended that sampling be extended to at least 72 h after treatment and that the highest possible dosages (e.g., 80% of the 7-d LD_{50}) be used. If the test is conducted by a less sensitive procedure, some mutagens may not be detected. In the tests conducted at Edgewood, the highest dosage was about 25% of the LD_{50}. Three mice were treated per group by single intraperitoneal injection, and they were killed to determine results after only 24 h.

In our view, it is premature to reach a conclusion regarding the genetic toxicity of CHT. Although the mutagenicity tests conducted at Edgewood provided no evidence that CHT is mutagenic, they were not adequate to support the general conclusion that it is nonmutagenic. One can therefore make no statement about the possibility that CHT could pose a mutagenic hazard for man. For a thorough discussion of bases for determining the likelihood that a given chemical is mutagenic in humans, see the recent report of the National Research Council Committee on Chemical Environmental Mutagens.[8]

EFFECTS ON HUMAN SUBJECTS AT EDGEWOOD

In late 1969 and early 1970, 16 subjects each underwent one experimental exposure to CHT in an aerosol chamber at Edgewood. The duration of exposure among the 16 subjects ranged from 30 s to 5 min. Ct's ranged from 15.4 to 64 $mg \cdot min/m^3$.

The effects of exposure on the subjects were transient, with complete resolution by 15 min after leaving the chamber. The predominant effects were lacrimatory, causing incapacitation due to eye closure and blurred vision lasting several minutes after exposure. Dermal irritation and rhinorrhea occurred. One subject had "chest congestion."

Laboratory analyses were performed 9 d after exposure to CHT. Two subjects had slight increases in SGOT after exposure (31.5 and 44.5 IU), representing slightly less than a doubling of their preexposure control values. SGOT was normal in both 1 mo later. One subject had a slight persistent increase in alkaline phosphatase 9 and 15 d after exposure (13.7, 14.3 KA), representing an increase to approximately 1.5 times his pre-exposure control value. Other tests of liver function performed on the subject with increased alkaline phosphatase 21 d after exposure had normal results. One subject had a decrease in hemoglobin content, from 16.8 g before exposure to as low as 13 g after exposure. A decrease in hematocrit from 48% to 44% accompanied the decrease in hemoglobin, and reticulocytes were 0.8% after exposure. Results of hemoglobin analyses from 9 to 20 d after exposure ranged from 13 to 14.2 g in the subject whose hemoglobin decreased, but his white-cell count was normal. These subjects were exposed to no other chemicals at Edgewood before the postexposure laboratory analysis. The slight abnormalities in SGOT after exposure to CHT might have represented idiosyncratic hepatic reactions to the chemical. Complete recovery is likely. The increase in alkaline phosphatase and the decrease in hemoglobin after CHT exposure are difficult to relate to the exposure.

Given the available information on CHT and on the Edgewood subjects exposed to the chemical, long-term health effects of the exposure on the subjects are difficult to predict.

SUMMARY

CHT appears to be a powerful lacrimator and irritant with a high safety factor and a short recovery time. It is highly volatile and dissipates rapidly in open air or in ventilated enclosures.

CHT appears safer than CR or CN. Neuromuscular damage in one dog from degraded CHT led to further studies, which demonstrated severe eye and skin damage from some of the degraded products, including neurologic signs, but no permanent damage in surviving animals.

Data are insufficient to support a conclusion regarding the mutagenicity or carcinogenicity of CHT.

TABLE 4-40

Comparative Data on CS, CR, and CHT[a]

Characteristic	CS	CR	CHT
Volatility at 20°C, mg/m^3	0.36	0.63	8,484[b]
ICt$_{50}$ for man, mg·min/m^3	6.9	0.15	ca.50
LCt$_{50}$, mg·min/m^3:			
Guinea pig	36,439	169,000	176,000
Dog	29,748	57,171	63,322
Percutaneous LD$_{50}$ for dogs, mg/kg	No data available	No data available	708-1,000[c]

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[a] Data from Simmons et al.[9]
[b] Volatility measured at 25°C.
[c] Animals held in room approaching saturation for 6 h.

TABLE 4-41

Effects of CHT Administered Cutaneously in Nonrestrained, Clipped Beagles Decontaminated at 24 hours with Bleach and Water[a]

Sample Composition,[b] mol%	Condition	Dosage ml/kg	Results
"Control"-- fraction 5 (88.2% EA 4923, 9.4% EA 4922, by NMR)	Occluded	0.125	2/2 normal at 24 h
		0.25	2/2 normal at 24 h
		0.50	1/2 dead at 2-3 d; 1/2 sacrificed at 5 d
Nonvolatile residue from 31AS in DMSO	Occluded	0.25	2/2 normal at 24 h
		0.50	2/2 normal at 24 h
		1.0	2/2 atactic at 2-3 d, but normal at 5 d
31AS sample (77.6% EA 4923, 11.7% EA 4922)	Occluded	0.125	2/2 normal at 24 h
		0.25	1/2 normal at 24 h; 1/2 unable to stand, convulsing at 24 h, and sacrificed at 2 d
		0.50	1/2 dead at 30-46 h; 1/2 with salivation, tremors, postural instability, but normal at 13 d
"Control"-- fraction 9 (85.1% EA 4923, 10.3% EA 4922), stabilized with 1% antioxidant	Occluded	0.25	1/2 dead at 2-3 d; 1/2 with tremors, emesis, postural instability, but normal at 7 d
		0.50	1/2 dead at 4-5 d; 1/2 with tremors, weakness, emesis, postural instability, but normal at 7 d
		1.0	2/2 dead at 1-3 d
"Control"-- fraction 8 (89.4% EA 4923, 8.4% EA 4922)	Nonoccluded	0.25	2/2 tremors, but normal at 2 d
		0.50	2/2 tremors, but normal at 2 d
		1.0	1/2 tremors, salivation, weakness, but normal at 5 d

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[a] Data from Simmons *et al.*[9]

[b] EA 4923 = CHT. EA 4922 = relatively inert isomer of CHT.

TABLE 4-42

Toxicity of CHT Administered to Skin without Occlusion in Nonrestrained Beagles Held in Saturated Environment[a]

Time in Environment	Dose ml/kg	Mortality fraction	Survivor Recovery Time,[b] d
1.5 h:			
Nonstabilized	0.5	0/2	6, 6
	1.0	0/2	N.E., 28
Stabilized	0.5	0/2	N.E., 6
	1.0	0/2	6, 20
6 h:			
Nonstabilized	0.5	1/4	6, 7, 23
	1.0	3/4	5
Stabilized	0.5	0/4	N.E., 2, 7, 13
	1.0	2/4	4, 19

---

[a] Data from Simmons _et al._[9]

[b] N.E.=neuromuscular effect such as tremors, weakness or ataxia noted.

TABLE 4-43

Effect of Decontamination with Bleach and Water or with Soap Solution and Water at Various Intervals after Skin Application of CHT at 1.0 ml/kg to Nonrestrained Beagles[a]

Sample	Decontaminant	Decontamination Time, min	Results
"Control"--fraction 4 (87.5 mol%)	Bleach followed by water	5	1/1 no toxic signs noted
		30	1/1 slight tremors at 3-22 h, but normal at 4 d
		60	1/1 dead at 30-72 h
"Control"--fraction 4	Soap solution followed by water	5	1/1 no toxic signs noted
		30	1/1 slight tremors,but normal at 4 d
		60	1/1 dead at 23 d
"Control"--fraction 4	Bleach followed by water	45	1/1 tremors, weakness at 1-19 d, but normal at 19 d
		60	1/1 tremors, weakness, postural instability at 3-25 d, but normal at 28 d
"Control"--fraction 4	Soap solution followed by water	45	1/1 found drowned in water pan on day 4
		60	1/1 tremors, weakness, postural instability at 3-25 d, but normal at 25 d
"Control"--fraction 9, stabilized with 1% antioxidant	Bleach followed by water	75	1/1 dead at 22 d
"Control"--fraction 8	Soap solution followed by water	75	1/1 tremors, weakness at 2 h-11 d, but normal at 11 d

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[a] Data fom Simmons et al.[9]

TABLE 4-44

Parenteral Toxicity of CHT in Rat, Mouse, and Rabbit

Species	Route[b]	LD$_{50}$, mg/kg
Mouse	Oral	478 (412-555)
Mouse	Intraperitoneal	13 (12-15)
Rat	Oral	282 (246-324)
Rat	Intraperitoneal	21 (16-27)
Rat	Intravenous	13 (11-15)
Rabbit	Intravenous	8 (7-9)

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[a] Data from Biskup *et al*.[1]

[b] Administered in polyethylene glycol 200.

TABLE 4-45

Toxicity of CHT Administered Cutaneously to Dogs[a]

Amount Applied,[b] ml/kg	Time, h	Acute Signs	Acute Mortality
1.0 (O)	1	1/8 (neurologic)	0/8
0.5 (O)	16 or 24	Not recorded	8/8
0.5 (O)	2	No toxic signs[c]	--
0.5 (O)	4-6	Toxic signs[c]	--
0.5 (O)	1 every 4 d for 4 doses	1/3 (neurologic)	0/3
0.25 (O)	1 every 4 d for 4 doses	0/3	--
0.125 (O)	1 every 4 d for 4 doses	0/3	--
0.5 (N)	24	0/4	0/4

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[a] Data from Dilley *et al*.[3]

[b] O = occluded, N = nonoccluded.

[c] Number of animals not stated.

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EFFECTS OF HUMAN EXPOSURES TO 123 IRRITANT CHEMICALS AT EDGEWOOD IN TWO-MAN TESTS

RL SOP No. 70-3, dated June 1, 1967, describes methods used at Edgewood for searching for and selecting toxic chemicals. Some of the details in connection with exposure of human volunteers to experimental irritant chemicals are described. Human volunteers were exposed to compounds after review of animal screening data and approval by committees based on a conclusion that the experimental chemicals were safe for human use. Generally, two volunteers were exposed to each substance. Subjects were exposed in a wind tunnel at an airspeed of 5 mph and were asked to resist leaving the test atmosphere (up to 1 min) until exposure was unbearable.

During 1962-1972, 123 irritant chemicals were tested. Further details on the chemicals are available from the NRC repository of Edgewood data. The substances were classified as irritants on the basis of the preliminary animal studies. Except where noted below, exposures were for 1 min or less in an aerosol chamber; each subject was exposed to a chemical only once.

AGENTS THAT CAUSED SLIGHT OR NO EFFECTS

Of the 123 irritant chemicals, 64 caused slight or no effects on the exposed subjects. Two subjects were exposed to each of the 64 chemicals, except CS 40806, to which only one subject was exposed.

The following 64 irritant chemicals caused slight or no effects:

301021
CS1086
CS4659
CS15442
CS20409
CS23653
CS27474
CS29780
CS30800
CS36650
CS36667
CS36722
CS37149
CS37200
CS38355
CS38731
CS39241
CS39242
CS39666
CS39715
CS40320
CS40325
CS40332
CS40679
CS40683
CS40686
CS40781
CS40785
CS40804
CS40805
CS40806
CS40841
CS40850
CS41462
CS41468
CS41592
CS41623
CS41725
CS42055
CS42057
CS42213
CS42216
CS42740
CS42824
CS43000
CS43001
CS43013
CS43014
CS43166
CS43168
CS43169
CS43945
CS43974
CS43988
CS43989
CS44854
CS45514
CS45659
CS46345
CS47137
CS47148
CS47563
CS48418
CS61804

CS38731 might have been contaminated with dioxin. Despite a lack of complete toxicity data, it seems unlikely that the short, single exposures to the 64 irritant chemicals that caused slight or no acute effects on the exposed subjects will cause long-term health effects.

LACRIMATORY AGENTS

The predominant effects of 42 of the 123 irritant chemicals were ocular, including eye irritation, eye closing, lacrimation, and conjunctivitis.

Of the 42 lacrimatory agents, 34 caused very mild effects--generally eye irritation, sometimes associated with dermal and upper respiratory passage irritation. The 34 mild lacrimatory agents were tested on two subjects each, except 119135 (four subjects), CS46398 (one subject), EA 2542 (17 subjects), EA 3365 (17 subjects), EA 4922 (six subjects), and CS815799 (ylidineamine, 21 subjects). The mild lacrimatory agents include:

118055
119135
302049
CS2127
CS5146
CS5616
CS18692
CS30747
CS30748
CS30749
CS30785
CS30799
CS31979
CS37270
CS38756
CS40331
CS40849
CS41377
CS41722
CS43331
CS43981
CS46398
CS48861
EA 2284
EA 2302
EA 2305
EA 2329
EA 2413
EA 2433
EA 2542
EA 3176
EA 3365
EA 4922
CS815799

CS38756 and CS40849 might have been contaminated with dioxin.

Additional details are available on EA 2542 and CS815799. In 1963, two subjects underwent one exposure each to EA 2542 at Ct's of 39 and 57 $mg \cdot min/m^3$. No effects were described. In 1969, EA 2542 was tested further on 15 subjects, who had one exposure each. Ct's ranged from 29.8 to 65.8 $mg \cdot min/m^3$, but no exposure durations are available. The subjects experienced irritation of eyes, nose, throat, and periorbital sites. Results of postexposure laboratory analyses were normal.

In 1972, 21 subjects underwent exposure to CS815799. Seven subjects had aerosol exposures at a Ct of 50 $mg \cdot min/m^3$; one was exposed twice. CS815799 exposure caused mild ocular and respiratory irritation. Laboratory analyses 7 d after exposure revealed increased SGOT (43.8 and 42.7 IU) in two subjects, both of whom had normal pre-exposure SGOT. One of these two subjects also had 6-10 white cells in urinary sediment--a finding not seen in preexposure urinalysis.

Fourteen CS815799 subjects were exposed cutaneously with 1% solution, which caused mild irritation. One subject was exposed twice.

Given the absence of followup data, it is not possible to predict whether short exposures to the 34 mild lacrimatory agents will have long-term health effects among the exposed subjects. The increase in SGOT in CS815799-exposed subjects might have represented hepatic reactions to the chemical, but recovery was probably complete.

Eight of the 42 lacrimatory agents caused more severe effects than the other 34, namely more prolonged incapacitation in association with lacrimation and eye closing: 118539, 123175, 126312, CS encapsulated in gelatin, CS36579, FOGOILCS, EA 2366, and CS-DM mixtures.

The CS-DM mixtures were tested on 88 subjects. The CS:DM ratio in the mixture was 1:10. Thirty-eight CS-DM exposures occurred in 1958, and half the 1958 subjects had two exposures each. Fifty of the CS-DM exposures occurred in 1966. Ct's were 0.5-40 mg·min/m^3. Exposure durations, when recorded, were 16 s to 2 min. The principal effects were ocular irritation, lacrimation, and conjunctivitis, sometimes associated with upper respiratory tract irritation and cough. One subject required analgesic therapy after exposure because of severe discomfort. Results of laboratory analyses 7 d after exposure, were available for all the 1966 CS-DM subjects, as well as some of the earlier subjects. One subject had an increase in SGOT (to 60 IU) in postexposure laboratory analysis, but his pre-exposure SGOT had also been slightly high (33.6 IU).

In 1963, two subjects underwent short, single exposures to 118539, which caused lacrimation and severe conjunctivitis, salivation, nasal irritation, chest constriction, and cough. In 1965, eight subjects underwent single exposures to 123175, which caused lacrimation and eye closing for several minutes after cessation of exposure. In 1966, 12 subjects underwent single exposures to CS36579, which caused eye pain, profuse lacrimation, eye closing and incapacitation that lasted several minutes after cessation of exposure, nasal irritation, and dyspnea. In 1963, two subjects underwent short, single exposures to EA 2366, which caused lacrimation and conjunctivitis, respiratory tract irritation and dyspnea, and nausea.

Given the available information on subjects exposed to 118539, 123175, 126312, CS36579, and EA 2366 and their short, low-dose exposures, one cannot predict long-term health effects of these agents. The discomfort associated with the exposures was marked, but exposures were short and recovery appeared complete.

RESPIRATORY IRRITANTS

Seventeen of the 123 irritant chemicals caused predominantly respiratory effects, which were generally mild and transient. Ten caused nasal and throat irritation, including cough. These upper respiratory irritants were tested on two subjects each, except CS5635, CS42818, and EA 2129, which were tested on four subjects each. Other upper respiratory irritants were CS24302, CS41458, CS42822, CS42984, CS43010, CS43109, and EA 3437.

Seven respiratory irritants caused primarily a sensation of chest constriction and dyspnea. EA 2097 (CS14632) was tested on 19 subjects, 15 of whom had two exposures each, and EA 2214 was tested on four subjects. Other lower respiratory irritants were tested on two subjects each: 118609, 119400, CS36273, CS42985, and CS43329.

Given the lack of followup information on the primary respiratory irritant chemicals, it is not possible to predict whether they will have long-term health effects. Because these were short, low-dose exposures in which the acute effects were generally mild, with complete recovery, the Committee believes that long-term health effects are unlikely.

CONCLUSIONS

The Committee analyzed published studies describing the in vivo and in vitro properties of the agents used and reviewed short-term data collected by the U.S. Army on volunteers. The ability to provide definitive answers to the questions raised by the charge to the Committee was limited by the absence of long-term followup studies of the soldiers and by the sparseness of chronic studies of these compounds in animals or in humans after industrial exposure.

In general, the Committee found insufficient evidence to evaluate these chemicals, except mustard gas. Mustard gas is an experimental mutagen and human carcinogen at high doses. Data on the other irritants are insufficient to evaluate their mutagenicity, carcinogenicity, or other long-term effects. Tests of all these chemicals involved few exposures and low doses.

MUSTARD GAS (H)

Mustard gas is highly reactive and has vesicant and systemic toxic effects. It is an alkylating agent that is mutagenic in various laboratory test systems, including mammalian germ cells, but data are inadequate to predict the extent of its genetic risk in humans. Mustard gas is also carcinogenic in experimental animals and humans. Other possible long-term effects of mustard gas are related to its systemic toxicity; specifically, it can cause blindness, permanent scarring of the skin (which may lead to skin tumors), and chronic bronchitis. Reported instances of long-term injury such as carcinogenesis in workers in a Japanese mustard production plant, were associated with exposure at high, long-term dosages. Information is insufficient to project risks associated with smaller exposures to mustard gas; however, serious long-term adverse effects in the small number of soldiers who received one or a few low-dose exposures at Edgewood seem unlikely (except for some cases of permanent scarring). Some of those exposed at Edgewood suffered skin injuries that took several weeks to resolve. However, in view of the small number of persons tested (about 150 healthy men) and the very low dosages involved, it is unlikely that a statistically significant increase in the risk of cancer or other chronic disease can be detected in those exposed to mustard gas at Edgewood. When exposed, the Edgewood subjects were wearing gasmasks and impregnated clothing--an ensemble being tested for efficacy against toxic contamination.

o-CHLOROBENZYLIDENE MALONONITRILE (CS)

Results of experimental studies in microorganisms and short-term experiments in laboratory animals suggest that long-term medical abnormalities in soldiers exposed to CS are unlikely. Acute tissue changes produced in animals and humans seem reversible and not likely to become chronic in the absence of recurrent exposures. Followup information on the long-term state of health of exposed soldiers is not yet available, but no reports have indicated that Edgewood subjects have experienced any long-term sequelae.

CHLOROACETOPHENONE (CN)

CN, a moderately toxic irritant, has immediate effects on the eyes, skin, and respiratory tract. CN is a strong skin-sensitizing agent, but is rarely lethal. The Committee found no evidence of lasting ocular or respiratory effects in 99 volunteers exposed experimentally at Edgewood between 1958 and 1972 when subjects were evaluated 2 wk after cutaneous administration or inhalation of aerosol. Allergic contact dermatitis or hypersensitivity in these volunteers on re-exposure to CN is possible. There has been no systematic study of the possible mutagenic and neoplasm-promoting effects of CN with current scientific methods.

DIBENZ[b,f][1,4]OXAZEPINE (CR)

CR, a mild lacrimatory irritant, manifests less acute toxicity than CN and CS. At low doses, it causes transient effects. There are a few studies on long-term health effects, including potential mutagenicity and teratogenicity. The available data are insufficient to predict long-term health effects. The small number of exposures and the small number of subjects exposed to CR at low doses at Edgewood make the occurrence of demonstrable effects in these subjects unlikely.

CHLOROPICRIN (PS)

PS is acutely toxic and has a variety of sensory effects in animals. It has not been evaluated thoroughly for mutagenicity or carcinogenicity. Like those exposed to mustard gas, the subjects exposed to PS were wearing gasmasks, and small numbers of soldiers were exposed to small doses. PS is unlikely to have produced detectable long-term health effects in volunteers exposed at Edgewood.

BROMBENZYL CYANIDE (CA), DIPHENYLAMINOCHLORARSINE (DM), and 1-METHOXY-1,3,5-CYCLOHEPTATRIENE (CHT)

CA, DM, and CHT are unlikely to have produced measurable long-term health effects in volunteers exposed at Edgewood. But there are no specific toxicologic data on the mutagenicity and carcinogenicity of these compounds. CHT is less toxic than CN or DM when administered acutely.

NONANOYL MORPHOLIDE

The Committee does not expect long-term health effects in volunteers tested with nonanoyl morpholide at the dosages used at Edgewood. As with CA, DM, and CHT, specific toxicologic data regarding its potential in this regard are not available.

123 IRRITANT CHEMICALS

A total of 123 irritant chemicals were generally tested on only two subjects each. There are no data on their mutagenicity, carcinogenicity, or other long-term health effects. However, because the exposures were small, detectable adverse effects seem unlikely.

APPENDIX A

Part 1. Reproduced from Volume I, Anticholinesterases and Anticholinergics

HISTORY OF THE EDGEWOOD TESTING PROGRAM

INTRODUCTION

Human experimentation appears to have been an integral part of the history of the U.S. Army chemical warfare (CW) research efforts until its suspension in 1975. On June 28, 1918, the President directed the establishment of the Chemical Warfare Service (CWS). Four years later, in October 1922, the CWS created a Medical Research Division to conduct research directed at providing a defense against chemical agents. No matter how exhaustively an agent was tested in animals, it was felt that its efficacy in humans also had to be studied.

In early 1941, the threat of war increased the urgency of the development of protection against CW agents and, consequently, engendered a need for a larger source of volunteers. Formal authority to recruit and use volunteer subjects in CW experiments was initiated in 1942. The Secretary of War was asked to rule on the permissibility of using enlisted men for testing agents of the mustard-gas type. In July 1943, the CWS was assigned responsibility for all medical research related to CW. This extension of the CWS mission included toxicological research and the study of hazards to the health of personnel in the CWS.

The issue of the use of human volunteers was considered by the Armed Forces Medical Policy Council during the early 1950's. The Council concluded that essential data could not be obtained unless human volunteers were used, and the use of humans in medical research was authorized. By 1954, the Chemical Corps (formerly CWS) had established a framework within which to conduct human experimentation, but it lacked an adequate pool of volunteers. In 1955, it was decided that the most practical source of volunteers would be enlisted men stationed at Army installations in the vicinity of Edgewood Arsenal. It was emphasized that voluntary consent of each human subject was absolutely essential. It was also stated that, in all experiments involving volunteer subjects, the subjects would be thoroughly informed of all procedures and of what might be expected as a result of each test. Furthermore, each volunteer would be free to determine whether he desired to participate in a given experiment. In October 1959, approval was granted for the conduct of research on volunteers to investigate defense against incapacitating CW agents.

The search for incapacitating agents intensified when the Kennedy administration took office. The involvement with incapacitating agents represented a departure from an earlier period, begun in 1946, when interest in highly toxic (acute) anticholinesterase chemicals resulted from their development in Germany during World War II. The basic purpose of a military incapacitating agent is to produce temporary ineffectiveness without permanent injury or death. Incapacitating agents (anticholinergic chemicals) and highly toxic (acute) anticholinesterase chemicals produce functional and structural effects on the nervous system which cause rapid or delayed effects on an individual's performance and behavior.

PROCEDURES USED AT THE EDGEWOOD CHEMICAL TESTING PROGRAM

A fairly extensive discussion of the procedures used is provided in the Inspector General's report, Use of Volunteers in Chemical Agent Research, prepared by Colonel James R. Taylor and Major William H. Johnson and dated March 1976 (listed in Appendixes C,D).

RECRUITMENT OF VOLUNTEERS

Recruiting teams (initially administrative officers, but later often including military physicians from the Edgewood laboratory) visited Army installations where a briefing, usually with a film and handouts, was presented to a large number of enlisted men. Generally 10 to 20 percent of the audience expressed interest and these men were asked to complete a personal history, which included medical and psychologic items and the Minnesota Multiphasic Personality Inventory (MMPI). It was not unusual for 400-600 men to request assignment in the course of a tour of seven to ten installations. Of these, no more than 100 were selected and eventually assigned for a 1- to 2-month period of temporary duty at Edgewood Arsenal.

The "incentives" for volunteering consisted of a small monetary allowance (approximately $1.50 a day for temporary duty), the assignment of only light duties while at Edgewood, and almost every weekend free. Some volunteers were genuinely interested in the scientific and experimental aspects; however, if curiosity or the desire to "test one's self" seemed too strong, the applicant was usually not accepted.

As a group, the volunteers were above average in physical and mental qualifications, with a mean IQ near 110, good behavior records, deviations of the population mean on all scales.

GUIDELINES FOLLOWED IN THE PROTECTION OF SUBJECTS

The Nuremburg and Helsinki guidelines were regarded by the investigators and their supervisors as appropriate constraints in studies performed on volunteers, although this was not clearly articulated in official memoranda until the mid 1960s. The provision of accurate, informative explanations of what was planned and what might be expected was regarded as essential to the continuance of the program. Written consents, witnessed by medical staff members, were required from the outset and became more elaborate with time. However, minutes of hearings conducted by the U.S. Senate Subcommittee on Health and Subcommittee on Administrative Practice and Procedure, September 10-12, 1975, stated that the consent information was inadequate by current standards.

INVESTIGATORS

When BZ studies were begun in 1960, the need for a psychiatrist with biologic training and interest was recognized and one was assigned to the program in January 1961. Physicians trained in internal medicine, anesthesiology, cardiology, surgery, dermatology, ophthalmology, neurology, and other specialities were assigned as the program proceeded. Many were research-oriented and have since gained excellent reputations in academic medicine at leading universities.

SELECTION OF DOSES FOR HUMAN TESTS

Subthreshold doses based on estimates from animal potency studies were used in the first few subjects. For example, the earliest exposures to BZ, one of the anticholinergic test compounds, were at doses between 0.1 and 0.5 μg/kg, which was less than one-tenth the incapacitating dose (ID) ultimately established at approximately 5.5 μg/kg. The intravenous route was preferred initially, but other routes of administration were also used. Inhalation studies were sometimes undertaken after a compound had been thoroughly studied by one of these parenteral routes. Oral and percutaneous studies were performed when effectiveness via these routes was of interest.

As the program developed, it became customary to test agents at dose increments of 40 percent, once the approximate effects of the lower doses were known. Placebos were used in some studies, but the cost with respect to subject confinement time, staff workload, and delay in achieving estimates of potency made this impractical except in special casses (e.g., evaluation of antagonists). Instead, low and high doses were assigned in a randomized manner by someone not involved in an experiment. Placebo responses were minimal. Signs of drug effects at all but the lowest doses were significant and made the value of placebo or "no treatment" inconsequential.

RANGE OF DOSES

Rarely did the intramuscular or intravenous doses exceed 1.5 times the incapacitating dose. Inhalation doses were higher, but potencies were lower by this route (usually about 60 percent of that by the intravenous or intramuscular route). Compared with doses described in the scientific literature on atropine coma therapy [18-23] or scopolamine therapy,[19] the BZ doses to which volunteers were exposed appear modest. As much as 20 times the ID_{50} of atropine and 30-40 times the ID_{50} of scopolamine have been administered in the past by clinicians--often to older and less robust patients. Many patients received multiple exposures of this magnitude over a period of days or weeks. These therapeutic procedures, reported several decades ago in refereed journals, actually stressed and advocated the benefits of such treatment, despite occasional deaths (most of which appear to have been caused by hyperthermia).

SAFETY MARGIN

The safety margin of a drug is defined as the ratio of the lethal dose (LD) to the effective dose (ED). Sometimes, ratio of the LD_{50} to ED_{50} is used, although a more conservative approach favors the use of the ratio of LD_1 to ED_{99} (standard margin of safety). In the case of incapacitating agents, much reliance is placed on extrapolation from animal experimentation, and estimation of the LD_1 is generally unreliable.

Many other extrapolation techniques have been used in manipulation of animal lethality data in an effort to generate a reasonable human estimate. By taking a conservative approach with data on deaths at low doses, one can derive estimates for man that are modest and in keeping with clinical judgement. Such methods depend on procedures developed and applied in toxicology.

APPENDIX A

Part 2. Clinical Research Department, SOP No. 5, August 12, 1968 (Revised)

VOLUNTEER SCREENING AND SELECTION

The purpose of this SOP is to provide guidelines for the psychological/psychiatric selection of volunteers. There are several standard forms used for this purpose and each will be discussed.

1. Screening Data form (medical history). A "yes" answer on any item without a recommendation of a medical officer for acceptance will reject the individual.

2. When the GT score is available a very low score (below 90 or 80) will reject the individual.

3. MMPI. These are "rules of thumb." Lacking a scientific basis for choosing, these represent advice rather than dogma, but should be followed if possible.

    A. Clinical Scales (Hs, D, Hy, Pd, Mf, Pa, Sc, Ma and Si)

        1. Overall profile. Reject if any five of the above scales are over 65.

        2. Mark profiles borderline and carefully examine family history for indication of psychological problems if---

            a. L and K both exceed F by at least 15 scale points.

            b. F exceeds both L and K by at least 15 scale points.

        3. Pd, Pa, Sc Pattern (psychoticism)

            a. Reject if any two of these three are among the two highest scores on the clinical scales.

            b. If Pa or Sc is above 80, and mark as borderline if either exceeds 70.

            c. Reject if Pa and Sc are both above 65 and are also both above Hs, D, and Hy.

4. Pd, Mf, Ma Pattern (Sociopathic Deviate; "Acting Out")

   a. Reject if Pd and Ma are both above 65, and there is a history of "acting out."

   b. Reject if Pd is above 70, and there is a history of "acting out."

   c. Reject of Mf is above 80 in combination with Pd, Pa, or Sc above 65.

5. Hs, D, Hy, Pt, Si Pattern (neuroticism)

   a. Reconsider overall picture, history, etc., if any four of these are above 70.

   b. Reconsider overall picture, history, etc., if any two of these are above 80.

   c. Reconsider overall picture, history, etc., if Pt is above 80.

The most common exception to these rules is the active, ambitious, college graduate with Pd and Ma above 65, but no history of acting out. In all but the most extreme cases it is well to obtain corroborating evidence from the Family History.

4. Family and Developmental History. The Family History (SMUEA Form 6-85) contains information about a wide range of the potential volunteer's activities, as well as tapping various levels of consciousness. For routine screening certain items are useful.

   1. Trouble in school, with the civilian police, or Article 15s. A pattern of this sort is indicative of an "acting out" type of person.

   2. Interviews with a psychiatrist for anything other than routing screening, e.g., peace corps selection, etc.

   3. A history of fighting after heavy drinking, especially with a bad temper.

   4. Blatant and diffuse expressions of hostility on the Picture Frustration Test pp 15-16.

Other items: slow rate of promotion, lack of clear cut goals, excessive depreciation of self value, and generally bizarre answers on the Sentence Completion may be clues but must be interpreted to relation to other information available.

The screening on the basis of the MMPI is usually done by the psychologist or psychiatrist. The Family Histories are read by the Medical Officers in Psychopharmacology with each officer reading his share. During this phase of the screening only those histories that have survived the preceding steps are read. The purpose of this screening is to pick those volunteers chosen to come to Edgewood for any type of test.

After all this material has been read and the volunteers rated, a list is furnished the administrative office of about 80 first choice names and 80 alternates. These names are usually given to the administrative office by the tenth of the month which precedes the month they are to report to Edgewood.

When the volunteers arrive at Edgewood they are interviewed by the officers in the Psychopharmacology Branch.

At the time of the screening interview, on the basis of the interview, history, questionnaire (sentence completion and Picture Frustration tests) and MMPI scores a rating will be applied to each candidate to separate out the following groups and an entry will be made upon the Physical Examination sheet opposite the heading "Psychiatric" characterizing the candidates qualification for drug testing, as follows:

Rating	Qualification (on PE form)
A	OK for psychochemical testing
B	Low-dose psychochemicals only
C	No psychochemicals
D	Equipment only

The ratings are to be defined as follows:

A. No apparent or overt psychologic problems and no tendency to somatize or act-out intra-psychic tension. Many assets, few liabilities. Flexible. Good ego strength. Age - appropriate maturity and responsibility. A clear sense of identity. Such conflicts as are evidenced are few in number, situational, and usually conscious. Normal MMPI and Family History.

An exceptionally well adjusted candidate who impresses the interviewer by his flexibility and ease in handling anxiety and hostile or aggressive impluses should be rated A+. These men will be used for such psychochemical tests as are considered to be of greater than usual stress.

B. Adequate, flexible, good ego strength. Gets along fairly well. An appropriately mature and responsible person. History is good but may have one or more negative items such as: a very minor offense for which an Article 15 is given or a civilian arrest for a minor, non-occurring matter (symptomatic of immaturity). Minor personality distortions which do not interfere with optimum functioning. These men are not optimal candidates for psychochemical testing but the interviewer expects they would suffer at most negligible psychic trauma from experience with the effects of psychochemicals.

C. Any tendency to psychosomatic reactions or aggressive physical acting-out should drop a candidate at least to this group. These men are good cooperative subjects who, however, are not candidates for psychochemical tests but may be used for other drug tests. They may be somewhat dull or non-verbal, have obvious neurotic traits, immaturity, rigidity or other apparent liabilities, but with good reality assessment and no borderline or psychotic tendencies at present or at any time in past history does not include bizarre circumstances or severe and continued traumatization.

D. These men fall into the lower end of a scale of group whose characterization agrees roughly with those rated as C. Some definite emotional pathology is tolerated in this group as well as some bizarre or unusual responses on the questionnaire tests and borderline or aberrent scores on some MMPI scales. Numerous but minor offenses (2 or 3 Article 15). These men may be used for equipment testing and at the discretion of the responsible medical officer may be used for local drug testing but should not be subjected to any systematic drug. Hysterical, or schizoid personalities and any but minor tendencies towards somatization should drop a candidate to this group. Some men who arrive at Edgewood with diagnosible physical or emotional disorder may be allowed to participate in the program but with D rating and their participation in any particular test must be OK'd by the responsible physician.

Such men who are untrustworthy, sociopathic, grossly disturbed or pathologic or have criminal history or a history of recurrent, severe or recent psychotic episodes should not be selected as volunteers and if they arrive at Edgewood, should be returned to their home station. This decision should ordinarily be made during the initial screening upon the basis of severe distortion of MMPI scores or very bizarre or unappropriate items on the history or questionnaire tests.

Under no circumstances should this SOP be construed to supplant or replace the judgement of the medical officers in the selection procedures, who may deviate from these guidelines at their discretion. Deviation from the SOP may also be done in a systematic way if adherence would interfere with the accomplishment of a particular investigation, as, for example, a study of the effects of psychochemicals upon depressed subjects. But the conditions of such an experiment would demand an unusual attention to the safety and well-being of the volunteers selected.

APPENDIX B

DIGEST REPORT
OXIMES

by

J. Henry Wills

INTRODUCTION

The group of oximes that has been administered to human volunteers by or under the auspices of the Biomedical Laboratory of the Edgewood Arsenal at Aberdeen Proving Ground includes several salts of *N*-methylpyridinium-2-formyl oxime, *N*,*N*'-trimethylene-bis-(4-formylpyridinium oxime)bisbromide and -bischloride, *N*,*N*'-methyleneoxymethylene-bis(4-formylpyridinium oxime)bischloride, and the ketoxime diacetylmonoxime. Two of the widely used salts of the monopyridinium oxime are the chloride (pralidoxime chloride), referred to here as I, and the methane sulfonate (contrathion), referred to as II (or P2S). The bispyridinium bisoximes above are known, respectively, as trimedoxime bromide (III), trimedoxime chloride, and obidoxime chloride (IV). The ketoxime is known as DAM (V). The designations by Roman numerals are used hereafter for these oximes.

Other salts of *N*-methylpyridinium-2-formyl oxime are identified by the abbreviation 2-PAM followed by the common symbols for elemental anions (such as 2-PAM I for the iodide) or the names of organic anions (such as 2-PAM tartrate). Other salts of *N*,*N*'-trimethylene-bis-(4-formylpyridinium oxime) are identified by appending the designation for the anion to the abbreviation TMB-4.

These and other oximes were developed to be reactivators of cholinesterase that had been inhibited by organophosphorus anticholinesterase compounds;[1,4] they were considered initially and briefly to be complete antagonists of the toxic actions of these substances. This idea had to be abandoned when 2-PAM I was found[5,6] to be much more effective as an adjunct to atropine than as a sole therapeutic agent in antagonizing intoxication by organophosphorus anticholinesterase agents. Furthermore, 2-PAM I and I antagonized particularly the blockage of nicotinic cholinergic neuromuscular transmission at the motor endplate on skeletal muscle--an effect that can be reproduced to some extent with d-tubocurarine and other curaremimetic agents.

The efficacy of oximes as adjuncts to atropine in treating intoxication by anticholinesterase agents depends on both the agent and the oxime. For instance, I is potent as an adjunct to atropine in treating experimental animals intoxicated by sarin or VX, but is only mildly effective as an adjunct to atropine in treating intoxication by tabun[6,8] and almost completely ineffective in treating intoxication by soman,[9] whereas III is moderately effective as an adjunct to atropine in treating experimental intoxication by tabun. None of the oximes considered here is outstandingly effective as an adjunct to atropine in treating intoxication of laboratory animals by soman.[9,13]

The failure of these oximes to antagonize the alterations in normal function induced by soman has been attributed[14] to hydrolytic dealkylation of the phosphorus atom in the phosphonyl residue attached to the active center of cholinesterase; that results in an alteration in the electronic field around the phosphorus atom that renders oximate ions unable to sever the bond between the phosphorus atom and the serine residue in the active center of the enzyme. The aging reaction, identified first with DFP,[15] has been found to proceed particularly rapidly in the phosphonyl residue from soman on inhibited cholinesterase.[13,16] A large dose of I was found[17] to stop the aging process in experimental animals, but reactivated only a part of the cholinesterase that had been inhibited. The amount of I required for this purpose was so large (104 mg/kg intravenously) as to carry a high hazard of toxic action. Furthermore, there is some uncertainty about the importance of aging in the response of an organism to soman. For example, the repetitive depolarization of skeletal muscle fibers after a single indirect stimulus that follows a dose of soman was stopped by TMB-4 Cl_2 without detectable reactivation of cholinesterase in the vicinity of the motor endplate.[18] Similar results have been obtained with d-tubocurarine,[19] gallamine,[19] and piperidyl methylandrostanediol.[20] Crone[21] reported that d-tubocurarine chloride in vitro at 10^{-4} M completely prevented for 6 h the aging of red-cell acetylcholinesterase that was inhibited by sarin and that gallamine triethiodide at the same concentration markedly slowed the aging of similarly inhibited cholinesterase. Because the response of skeletal muscle after a dose of soman was affected by a dose of d-tubocurarine that would have yielded a concentration in the blood of no more than 0.45×10^{-6} M, it is difficult to believe that Crone's effect can explain fully the in vivo action of d-tubocurarine in antagonizing the neuromuscular blocking action of soman.

In 17 rats, twitch contraction of the anterior tibialis muscle in response to single indirect stimuli continued until a mean dose of soman had been given that was more than 2,100 times the minimal dose that altered the magnitude of the twitch.[22] Eventual failure of the twitch response was seen to be always connected with marked slowing of the heart. This observation raises the possibility that the effect of soman on the twitch response of skeletal muscle depends on a deficient supply of blood to the muscle, rather than on an effect on neuromuscular transmission. However, a similar indirect action seems not to explain the effect of soman on the response of skeletal muscle to repetitive indirect stimulation. Skeletal muscles become unable to maintain a tetanic response to repetitive indirect stimulation at doses of soman below those that affect significantly cardiovascular function. The indication that TMB-4 Cl_2 can antagonize the effect without inducing reactivation of cholinesterase at the neuromuscular junction[18] illustrates the importance of knowing what actions other than reactivation of cholinesterase may reside in the molecules of not only III but also the other oximes with which this review is concerned.

LETHALITY OF SINGLE DOSES IN EXPERIMENTAL ANIMALS

Several compilations of the toxicities of oximes have been published.[23-29] Additional information on the toxicities of the oximes under consideration is available from several sources, including Namba,[30] Lindsey et al.,[31] Wills,[32] and Crook and Cresthull.[33] Table 1 summarizes the available information on the single-dose lethalities of the oximes by giving typical values, without attribution to specific investigators. In comparing lethal doses of the various oximes, assuming that the oximate radical is the biologically active portion of the molecule, knowledge of the relative amounts of this radical in the various compounds is important. Table 2 gives values for this measure; the last column of the table gives values for the relative lethal activities of the compounds derived from the data in Table 1. It is obvious that there is no measurement in Table 1 on which all eight oximes can be compared, so that the relative lethality values in Table 2 should not be taken too seriously; they may be approximately correct in order. Lethality after oral administration was omitted from the consideration of relative lethality because it obviously differed qualitatively from lethality by other routes of administration; e.g., all five of the oximes given to mice orally were less toxic than 2-PAM I, although all were more lethal than 2-PAM I by any other route of administration. When the two rankings in Table 2 are compared by means of Kendall's rank correlation coefficient, there is only a 90% chance that the ranks in the two measurements are significantly correlated Thus, the relative amounts of the oximate radical in the molecules of the different oximes may not explain completely their comparative lethalities.

Seven of 14 dogs given single intravenous doses at 187 mg/kg of 2-PAM I died.[33] Vomiting, weakness, tremor, salivation, loss of reflexes, and convulsion were the most common signs of intoxication in these animals. Seven of 16 dogs given single intravenous doses of III at 57.5 mg/kg by the same investigators died. Weakness, convulsion, tremor, salivation, loss of reflexes, and vomiting were the most common effects. In general, the signs of toxicity appeared in approximately the following order after each oxime: staggering weakness, collapse, relaxation of muscles, urination, defecation, tremor, convulsion, gasping, salivation, loss of reaction to touch, sound, or pricking with a needle, loss of eye reflexes, apnea, cyanosis, and death. The principal difference in effects between 2-PAM I and III was that muscular weakness was a less immediate response after 2-PAM I than after III.

Cholinolytic drugs have been found[34,35] to increase the lethalities of II, III, and a 1:1 mixture of II and III in mice given intramuscular injections. The changes in the LD_{50}s of II and III induced by a constant dose of atropine were 17.9% and 17.8%, respectively, despite the fact that the LD_{50}s of the oximes alone differed by a factor greater than 2. Parpanit and several mixtures of cholinolytic drugs had effects qualitatively similar to those of atropine. Duke and deCandole[36] reported that intramuscular injection into rabbits of equal doses (30 mg/kg) of I, II, and III resulted in peak plasma concentrations of the oximes about 9 min after the injections, the peak concentration of III being considerably greater than those of I and II. These investigators reported also that, whereas the plasma concentrations of I and III after intravenous injections decreased more slowly than that of II, the plasma concentration of all three after intramuscular injections decreased at about the same rate. Inasmuch as I and II had similar peak blood concentrations (lower than that of III) after intravenous injections, I and II seem to have somewhat larger volumes of distribution in the body of the rabbit than III.

In the rat, absorption of III from a single-loop intestinal preparation during 1 h was found to be only about 13% of that of 2-PAM I.[37] The rate of absorption of I was somewhat lower than that of the iodide; II was absorbed at nearly the same rate as the iodide.[38] Three hours after the oximes were put into intestinal loops, slightly more than one-third as much of III had been absorbed as of 2-PAM I.

Brown[39] found that intracisternal injection of II, after injection of sarin by the same route, was ineffective in overcoming respiratory paralysis and vasomotor stimulation resulting from sarin, but that an intravenous dose of atropine was effective. Edery[40] extended this sort of study with several organophosphorus compounds, atropine, I, III, and V. He found that intraventricular atropine and, to a minor extent, oximes were able to antagonize the effects of intraventricularly injected ethyl pyrophosphate. Intravenous injection of V at 25 mg/kg 1-2 min after intraventricular injection of ethyl pyrophosphate did not modify the effects induced by the organophosphorus compound. Intravenous injection of III at 20 mg/kg or, especially, of 2-PAM I at 50 mg/kg had definite antagonistic effects

TABLE 1

Representative LD_{50} Values of Eight Oximes Administered to Seven Animal Species

		LD_{50}, mg/kg							
Species	Route[a]	2-PAM I	2-PAM Cl (I)	P2S (II)	2-PAM lactate	TMB-4 Br_2 (III)	TMB-4 Cl_2	Obidoxime (IV)	DAM (V)
Mouse	IV	133	155	--	122	44	--	130	--
	IP	210	140	--	--	60	110	139	68
	IM	230	180	231	--	80	130	160	--
	SC	257	222	165	--	83	--	183	--
	PO	1,650	2,590	3,700	1,920	2,000	--	3,390	--
Rat	IV	147	96	109	--	89	104	140	--
	IP	300	199	--	--	165	--	195	--
	IM	--	150	218	--	137	--	189	--
	SC	--	--	332	--	--	--	--	--
	PO	--	--	7,000	--	--	--	4,000	--
Guinea pig	IM	--	168	305	--	--	--	79	--
Rabbit	IV	--	94	133	--	--	44	83	--
	IM	--	--	245	--	--	--	--	--
Cat	IV	--	--	--	--	--	--	100	--
	IM	--	--	--	--	117	--	188	--
Dog	IV	190	--	--	--	60	--	70	--
Monkey	IM	--	--	356	--	--	--	--	--

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[a] IV, intravenous; IP, intraperitoneal; IM, intramuscular; SC, subcutaneous; PO, by mouth.

TABLE 2

Relative Oximate Content and Relative Lethality of Eight Oximes

Oxime	Relative Oximate Content	Relative Lethality
2-PAM I	1.00	1.00
P2S (II)	1.13	1.27
2-PAM lactate	1.17	1.10
TMB-4 Br_2 (III)	1.18	2.56
Obidoxime (IV)	1.47	1.39
TMB-4 Cl_2.	1.48	1.67
2-PAM Cl (I)	1.53	1.33
DAM (V)	2.61	3.13

on the responses induced by prior intraventricular injection of ethyl pyrophosphate. In interpreting these findings, it is pertinent to point out that the doses of the various oximes used correspond with 0.044 mmol/kg of III, 0.189 mmol/kg of 2-PAM I, and 0.248 mmol/kg of V. On the basis of these values, Edery's conclusion that 2-PAM I was the most active antagonist of ethyl pyrophosphate may be questionable.

These and other findings have raised the question of whether quaternary pyridinium oximes can enter the CNS from the general circulation. Kalser,[41] in a study with rats and cats given intravenous injections of I tagged with ^{14}C in the quaternizing methyl group, found that the cerebrum, the cerebellum, the medulla oblongata, and the spinal cord contained only traces of ^{14}C at times at which the blood contained the label at 19-53 μCi/kg. However, Firemark *et al.*[42] found that the brain of the rat had a concentration of ^{14}C-2-PAM I about one-tenth that in plasma 10 min after intravenous injection of labeled oxime at 20 mg/kg. In the brain, the cerebral and cerebellar cortices had the highest concentrations of the label; the caudate nucleus, the thalamus, and the hypothalamus contained concentrations a little less than half those in the cortices. Rats pretreated with the anthelmintic organophosphate trichlorfon and killed 10 min after intravenous injection of labeled 2-PAM I had a concentration of the label in their brains about twice that found in normal rats. The brain appears, therefore, to be somewhat permeable by 2-PAM I, but distinctly less so than leg muscle, diaphragm, liver, and kidneys according to Kalser's data, and to have that permeability increased by an organophosphorus anticholinesterase compound.

Freshly prepared solutions of II (150 mg/ml) in water or DMSO were applied to the skins of guinea pigs and rabbits, except that on the head and legs.[43] The II in DMSO entered the blood of the rabbit at a peak rate about 3 times that at which II in water penetrated the skin. No uptake of II from the aqueous solution by the guinea pig was detected. The rate of uptake of II from the DMSO solution by the guinea pig was about two-thirds that by the rabbit. Guinea pigs that had been anointed with the DMSO solution of II were given sarin subcutaneously at 200 ml/kg and immediately thereafter atropine sulfate intramuscularly at 15 mg/kg. Similarly anointed rabbits were given sarin intravenously at 100 μg/kg and then atropine sulfate intramuscualrly at 15 mg/kg. For the guinea pigs, the shortest interval between skin application of II in DMSO and sarin administration at which death occurred was 12 h. For the rabbits, the corresponding time was 5 h. It is apparent, therefore, that DMSO can facilitate the movement through guinea pig and rabbit skin of sufficient II to maintain protective blood concentrations of II for reasonable times.

The LD_{50} of I, injected intravenously, for rabbits was decreased by prior intravenous injections of d-tubocurarine at 0.1 mg/kg or atropine sulfate at 2 mg/kg by 63.4% and 9.3%, respectively.[44] Prior intravenous injection of neostigmine bromide at 0.1 mg/kg increased the LD_{50} by 31.2%. These findings indicate that I exerts its principal action on the neuromuscular junction in skeletal muscles and has only a minor effect on muscarinic cholinergic junctions. This conclusion agrees generally with that from Kalser's work. It is reinforced by reports that I blocks transmission in the isolated rat phrenic nerve-diaphragm preparation;[45] that it antagonizes the stimulant action of acetylcholine on isolated frog muscle;[46] that it decreases the response of the frog rectus abdominis muscle to decamethonium and to carbamylcholine, in addition to the response to acetylcholine, but in large concentrations increases partial blockade of transmission in the rat phrenic nerve-diaphragm preparation induced by prolonged exposure to decamethonium;[47] and that it increases, and in high concentrations decreases, the endplate potential.[48]

Wagley[48] found that V produced a dose-related decrease in the endplate potential of the curarized iliofibularis muscle of the frog at $1\text{-}3 \times 10^{-2}$ M. A similar effect of 2-PAM I was found at $1\text{-}3 \times 10^{-3}$ M, whereas concentrations below 10^{-3} M produced dose-related increases in the endplate potential. Fleisher _et al._[47] found that III, unlike I, had no excitatory action on the isolated frog rectus abdominis, but had a more potent effect than I in inhibiting the response of that muscle to decamethonium, carbamylcholine, and acetylcholine.

Kunkel _et al._[49] found that intravenous administration of III at 5 and 10 mg/kg had no effect on the response of the cat gastrocnemius-soleus muscle group to supramaximal indirect stimulation at either slow (0.5/s) or tetanizing frequencies. An intravenous dose of 20 mg/kg produced a marked temporary decrease in the response to a tetanizing frequency without altering the twitch response. An intravenous dose of 40 mg/kg almost abolished for 25 min or more the response to delivery of a tetanizing frequency to the motor nerve and decreased by about 80% the tension developed in the twitch response. That dose of III also blocked partially the response of the heart to stimulation of the vagus nerves, but did not modify the changes in blood pressure induced by bilateral occlusion of the carotid arteries or by intravenous injection of acetylcholine chloride or epinephrine chloride at 3 μg/kg. The effect of III, like that of I, seems to be predominantly on nicotinic cholinergic junctions. The effects of TMB-4 Cl_2 on the response of the cat heart to stimulation of the vagi and on contraction of the nictitating membrane after preganglionic stimulation of the superior cervical ganglion were more marked than those of the same intravenous dose (15 mg/kg) of I.[50]

Dultz et al.[23] infused V intravenously into dogs at 50 mg/kg per minute and I at 30 mg/kg per minute. The mean times to death after infusions of the two oximes were 10 and about 33 min, respectively. These times correspond with relative lethal doses of 100 and 196.5, respectively. These values are not far from those for intraperitoneal injections of the two oximes into mice.[100,206] After V, the heart rate rose initially and then, after about 3 min of infusion, began a slow decrease. The diastolic pressure decreased progressively from the start of infusion, whereas the systolic pressure remained fairly steady during the first 4 min of infusion and then began to decrease sharply. The rate of breathing and the tidal volume were reasonably constant during the first 5 min of infusion; the rate of breathing then increased, after an initial brief decrease, to nearly 3 times its original value by 9.5 min after the start of infusion. During the same period, the tidal volume decreased to about one-tenth its original value. At 10 min after the start of infusion, the animal became apneic, the pulse pressure fell to zero, there was a brief period of arrhythmia associated with an elevation of the J segment of the ECG, and then the dog died.

The most striking changes during the early minutes of infusion of I were increases in systolic and pulse pressures. These were accompanied by an increase in breathing rate without much change in tidal volume or heart rate. After about 28 min of infusion, systolic pressure and heart rate began to decrease. About 3 min later, both systolic and diastolic blood pressures fell precipitously with heart rate and tidal volume. Breathing rate had begun to decrease sharply after about 28.5 min of infusion. Terminally, the T wave of the ECG was increased and prolonged, and the voltage of the QRS complex was markedly reduced. Death followed apnea by only a few minutes. The changes reported by these investigators suggest that V kills by CNS depression, whereas I kills by interfering with repolarization and contraction of cardiac muscle.

Ballantyne et al.[51] gave rabbits intramuscular or intravenous injections of II at the LD_{50}. They found that plasma II maintained at less than 90 μg/ml was not lethal. Concentrations of 90-160 μg/ml were not lethal if they persisted for only a few minutes. If concentrations in that range were maintained for 40-50 min, there might be a sudden increase to above 200 μg/ml. A plasma II concentration above that limit generally led to death. The II concentration in the aqueous humor of the eye increased slowly, but an hour after injection was nearly the same as that in the plasma. Thereafter, the II concentration in the plasma and in the aqueous humor decreased at similar rates.

TOXICITY OF REPEATED DOSES IN EXPERIMENTAL ANIMALS

Rats and rabbits received intramuscular injections of solutions of II in saline 5 d/wk for 11 and 9 wk, respectively.[52] Dogs were given gelatin capsules of II by mouth 5 d/wk for 17 wk. Control groups of rats and rabbits were given physiologic saline intramuscularly on the same schedules as those which received II. No control dogs were included in the experiment; comparisons were made between the dogs given II and "normal" animals. The daily doses of II injected into rats were 50 and 150 mg/kg; those injected into the rabbits were 50 and 100 mg/kg. Dogs weighing 13-17 kg received daily doses of 1 g, or about 59-77 mg/kg.

The rats given II seemed to be normal both grossly and by microscopic examination of sections of tissues at the end of the study. The rabbits had no abnormality clearly attributable to II other than purulent, indurative myositis at the site of injection in nine of 10 animals. Inasmuch as the solutions injected were not stated to have been sterilized, the myositis is not astonishing. The stomachs of the three dogs all had fibrosed mucous membranes at the cardiac and/or pyloric regions. The plasmas of these dogs had subnormal concentrations of albumin and total protein and low albumin:globulin ratios.

The rats given either of the doses of II grew normally, and indeed somewhat more than the controls. The rabbits, as is not unusual, had coccidial infestations of their livers and intestines, but had no lesions attributable specifically to the oxime. The plasma II concentrations of the dogs shortly before they were killed at the end of the exposure period were around 60-90 μg/ml. One of the three dogs had roundworms (Toxascaris leonina) in its intestines and granulomatous nodules in its kidneys and pancreas due to this infestation.

The same investigators made a comparative study of the toxicities of I and TMB-4 Cl_2 in rabbits and dogs, published as an internal report by FOA 1 (C1024-F100) in April 1963 and as a paper in April 1964.[53] Groups of eight rabbits received intramuscular injections 5 d/wk for 12 wk of I at 65 mg/kg, TMB-4 Cl_2 at 30 mg/kg, or physiologic saline at 0.2 ml/kg. The solutions were sterilized by filtration through a Seitz filter. Groups of four young beagles (9-11 kg) were given capsules containing 1 g of TMB-4 Cl_2 or of I 5 d/wk. For TMB-4 Cl_2, this dose was continued throughout the 15 wk of the study; for I, the daily dose was reduced to 0.75 g after the first 2 wk and kept there until the end of the 15-wk study.

Except for local changes at the site of injection, the rabbits given I intravenously suffered no detectable toxic effect other than a minor loss of weight. Those given TMB-4 Cl_2 began to die during the third week of the study, six of eight rabbits having died by the end of the 15-wk period. The blood of the rabbits given either oxime was seen to clot rapidly, the effect being more marked after TMB-4 Cl_2 at 30 mg/kg than after I at 65 mg/kg and lasting for 4-5 h after an intramuscular injection. The dogs given capsules of TMB-4 Cl_2 had no signs of intoxication by the oxime, whereas those given capsules of I had diminished activity, ataxia, and head drop starting

2-3 h after they were given their capsules. After the daily dose of I was reduced, these signs of intoxication disappeared; they became evident again in three of the four dogs during weeks 10-12 of the study. The signs of renewed intoxication appeared on only a few days in each dog and then disappeared again, although administration of I continued. The curves of gain of body weight by the dogs were unaffected by the oximes, and all were judged to be in a normal state of nutrition at the end of the 15-wk period.

At the sites of injection of oxime in the rabbits, various extents of hemorrhage and of purulent, indurative myositis and muscle necrosis were seen. The rabbits that received physiologic saline and five that received intramuscular injections of 2.15 M sodium chloride on 8 d in a satellite experiment had waxy degeneration of muscle at the site of injection. Five rabbits that received intramuscular injections of 2.15 M I on 8 d also had waxy degeneration of muscle at the site of injection. This was stated to be more extensive than that seen in the rabbits given equimolar sodium chloride. No other lesions in the rabbits that seem to be attributable to the oximes were described.

The dogs given capsules of the oximes were found to have hyperemia in their stomachs and intestines. Four of the eight dogs had erosions of gastric mucosa, evident particularly in the apices of the rugae in the fundus and found in animals given I as well as in those given TMB-4 Cl_2. All the dogs had epithelial defects and proliferation of the connective tissue of the lamina propria. Atrophy of stomach glands was seen sometimes. The brains of both the dogs and the rabbits were described as having peculiar, slightly granular, basophilic structures in the white matter of the brain stem. These could appear as spheres or clouds. The figure purporting to demonstrate these structures does not do so clearly enough to permit a guess as to their nature; they may be nothing more than fixation artifacts.

Inasmuch as myositis was reported in the second paper[53] as well as in the first,[52] this response must be induced by the oximes and not by bacteria. The second paper showed that oral doses of I and TMB-4 Cl_2 induced the same sort of scar formation as II in the gastric mucosa, so that this response may be induced by either the oximino group or the quaternary nitrogen atom. It would be informative in this regard to have the results of an experiment in which capsules of pyridine-2-aldoxime and of N-methylpyridinium chloride were administered in a similar fashion.

The toxicities of repeated intravenous doses of I and II in rabbits and dogs have been estimated.[54] Six rabbits were given I at 50 mg/kg 5 d/wk for 6 wk; four rabbits were given II at the same dose on the same schedule. Three dogs were given I at 25 mg/kg b.i.d. 5

d/wk for 6 wk; three other dogs were treated similarly with II. During the 6 wk of the experiment, 91 observations of signs of toxicity were recorded for the dogs given II and 115 for those given I. In both groups of dogs, the most common observation was hyperventilation. This sign accounted for 60% of the observations recorded for the dogs given I and 78% of those for the dogs given II. The next most common sign for I was ataxia; for II it was vomiting. Collapse was the third most common sign with both oximes, but accounted for less than 6% of all the observed signs of toxicity. Tremor and jerking of the head were equally frequent signs of intoxication in fourth place with I, whereas tremor and ataxia had equal incidences in fourth place among the dogs given II. The only other sign reported was salivation, which accounted for less than 2% of the total of recorded signs. Hyperventilation, mentioned above as the most frequent sign of intoxication, was stated to occur only during injections of the oximes and to stop immediately after the end of the injection.

All the dogs given intravenous injections of an oxime had decreases in their blood concentrations of hemoglobin (mean, -23.2%). Two of the dogs given I had increases in their hematocrits, whereas only one of the dogs given II had this sort of change. All three dogs given I had decreases in their leukocyte counts, whereas two of the dogs given II had increases in their leukocyte counts. Of the four dogs that had decreases in their total leukocyte counts, two had increased percentages of polymorphonuclear leukocytes and two decreased percentages. The two dogs with increased percentages of PMN leukocytes had subnormal percentages of lymphocytes. The two dogs with decreased percentages of PMN leucocytes had increased percentages of lymphocytes.

Two rabbits died after the third dose of I, and two other rabbits from this group died during a weekend after having developed diarrhea that lasted 2-7 d. One rabbit given II died after the fourth injection. Another rabbit in this group developed diarrhea during the fifth week and died during the following weekend.

Three rabbits and three dogs given I and two rabbits and three dogs given II were subjected to necropsy at the end of the experiment. No lesions attributable to the oximes were found. Other rabbits housed in the same areas as those used in this study were reported to have developed diarrhea and in some instances to have died, so that the deaths of rabbits during the experiment may have been unrelated to the experimental procedures. The decreased hemoglobin concentration and the diminished white-cell counts in dogs may have been induced by the oximes, but the larger dose of II given to rabbits by Albanus et al.[52] during a longer period did not result in decreases in hemoglobin concentration or in the red-cell count, although it may have induced a decrease in the white-cell count. Crook et al.[54] concluded that I and II have relatively low toxicities for dogs and rabbits when they are given intravenously in daily doses of 50 mg/kg on 5 d/wk for 6 wk and that I is more likely to induce ataxia than II, but is less likely to induce vomiting.

A comparative study of the toxicities of I, III, and IV administered to rats by gavage and to dogs in capsules has been reported.[55] Groups of five rats were given daily doses of I, III, or IV at 20 mg/kg through intragastric catheters 5 d/wk for 4 wk. Wheezing was the most frequent observation, even occurring in the control group. Wheezing was observed more frequently among the rats given oximes than among the control animals; it was less frequent in the group given IV than in those given the other two oximes. Chronic inflammation of the lower respiratory tract was found in almost all the rats. Irritation of the eyes occurred in a few rats of each group, including the control group. Hematologic measurements, organ weights, body weights, food consumption, and gross and microscopic surveillance of organs and tissues at necropsy revealed no oxime-related changes.

The control dogs (given empty capsules on the same schedule on which other dogs received oxime-containing capsules) and most of those given oximes seemed to be in good health throughout the study. The exceptions were one dog in the group given I that exhibited fasciculations and tremors 10 min after receiving its first capsule, one dog in the group given III that retched 10 min after receiving its capsule on the twelfth day of the study, and one dog in the group given IV that retched about 4 h after being given its capsule on the ninth day of the experiment. No dogs died, and all gained weight at about the same rate as during a preliminary observation period. No alterations that seemed to be related to ingestion of the oximes were found in hematologic values, blood chemistry, urinalysis, organ weights, organ:body weight ratios, or gross and microscopic appearances of tissues and organs removed at necropsy.

An additional study of daily intravenous injection into rats and dogs of IV at 35 mg/kg 5 d/wk for 4 wk used groups of 10 rats and four dogs--two males and two females.[56] The only visible signs of toxicity observed in the rats were wheezing, hyperpnea, and "swelling in the throat." Wheezing, recorded 11 times during the total of 200 rat-days, was the most common sign among the animals given IV; it was recorded once in the control group and was the only abnormal observation for that group. "Swelling in the throat" was recorded four times among the rats given IV, and hyperpnea three times.

The mean consumption of food and the mean rate of gain of body weight were significantly lower in rats given IV than in the control group, which was not given sham injections. Hematologic measurements disclosed no significant differences between the two groups of rats. The mean liver and kidney weights were reduced in the rats given IV; the mean adrenal weight in these animals was identical with that in controls. When organ:body weight ratios were calculated, the ratios for liver and kidney in rats given IV did not differ significantly from those in the control rats. The ratio for the adrenals in rats given IV was above that for the control group. One concludes that the anorexia resulting from injection of IV into the rats affected the weights of the liver and kidneys in proportion to its effect on the overall body weight, but did not alter the size of the adrenals.

The only sign of toxicity recorded for the dogs given IV was retching, which was recorded six times during the 80 dog-days of observation within the second, third, and fourth weeks of the experiment, but not during the first week. The dogs given IV lost 2.34% of their original mean body weight during the experiment, whereas the control dogs lost only 1.68%. One bitch among the dogs given IV had increased alkaline phosphatase and transaminase activities in its serum after the fourth week of the experiment, but not after the second week; microscopic study of sections of this animal's liver revealed degenerative changes in the periphery of the lobules. The livers of the other three dogs in this group were free of significant pathologic findings, so that the changes in the liver of one of the bitches may have been unrelated to administration of the oxime. No other significant pathologic findings were reported, except for the finding of intestinal parasites (hook worms, round worms, tape worms, or whipworms) in most of the dogs. Hematologic, blood chemical, and urinanalytic studies revealed no significant differences between the control group and that given IV other than those mentioned above for one bitch.

The toxic effects of 2-PAM I and III on rabbits and dogs, given intravenous injections 5 d/wk for 6-8 wk, have been evaluated.[57] Both rabbits and dogs received daily doses of 2-PAM I at 30 mg/kg and of III at 10 mg/kg. Two dogs and two rabbits received intravenous injections of physiologic saline on the same schedule followed for injecting oximes. Groups of three dogs and three rabbits were given the oximes. All animals were observed for signs of toxic effect after the injections and were weighed weekly. Hematologic and blood chemical studies were performed on the dogs. Rectal temperatures of the dogs were measured once each week. All animals were subjected to necropsy at the end of the study. Samples of abdominal and leg muscles and of blood from the dogs were analyzed for oximes.

All rabbits gained weight during the 6-wk periods of injection of 2-PAM I (0.26-0.84 mg/kg) and of III (0.18-1.14 mg/kg). The dogs ate well and seemed to be in good physical condition during the 8-wk period of injection of 2-PAM I. Two dogs maintained their original body weights, and the third gained about 0.5 kg. The dogs neither lost nor gained weight during the 6-wk period of injection of III.

Neither the rabbits nor the dogs gave visible evidence of toxic effects during the experiment.

The only indicator that suggested an effect on the composition of the blood of the dogs was the white-cell count after 8 wk of injection of 2-PAM I. This increased by a mean of 27.6%, whereas it decreased by 5.5% in the control group during the same period. If the plasma concentration of an oxime 5 min after intravenous injection was taken as the initial value, at 30 minutes it had fallen to 21.8% (2-PAM I) or 28.3% (III) and at 60 min it had fallen further to 10.7% (2-PAM I) or 14.4% (III). It is apparent, therefore, that III is removed initially from the plasma somewhat more slowly than 2-PAM I, but that the difference is not great and that there may be an increase in the rate of removal of III somewhere between 30 and 60 min after the initial value. The percentages of the initial concentrations of 2-PAM I and III remaining in the plasma at 155 min after estimation of the initial values differed by only about 0.1%

Abdominal and thigh muscles examined 20 h after the last intravenous injection of oxime contained no detectable amount of either oxime. Samples of muscles collected 30 and 90 min after the last dose of oxime contained 2-PAM I at higher concentration than III. At both these times, thigh muscle contained a higher concentration of 2-PAM I than abdominal muscle, but a lower concentration of III. Dogs and rabbits appear, therefore, to tolerate repeated daily intravenous doses of 2-PAM I at 30 mg/kg or of III at 10 mg/kg during a period of 6-8 wk when the daily doses are suspended during each weekend. Because in this and the other studies reviewed the animals were killed at or soon after the end of the period of administration of an oxime, there has been no opportunity to judge whether repeated administration of an oxime may initiate some alteration in normal structure or function that will result eventually in a definite lesion. No truly chronic study of the toxicity of an oxime has been found. Thus, possible cryptic toxic effects of this type of compound have never been assessed.

SIDE EFECTS OF OXIMES IN MAN

Hopff and Waser[58] have listed mechanisms whereby reactivators of inhibited cholinesterase could be harmful to persons to whom they are administered in treatment of intoxication by anticholinesterase compounds. The following is a slightly modified version of their list:

- The reactivator may affect enzymes other than those involved directly in the actions of the inhibitor of cholinesterase.

- The reactivator may itself affect some part of the active center of cholinesterase.

- The reactivator may form, either with the inhibitor or with its residue in inhibited cholinesterase, a stable secondary inhibitor of cholinesterase.

- The reactivator or a stable complex between the reactivator and the inhibitor may affect important functional systems of the body other than those related to cholinesterase.

- The reactivator may be metabolized to harmful products.

The last three mechanisms of action, and possibly the other two as well, are involved in the causation of the side effects that have been reported to occur either in normal subjects to whom reactivators had been administered during research projects or in patients who had been given reactivators as therapy for intoxication by pesticidal anticholinesterases or other inhibitors of cholinesterases.

Some side effects seem to be common to all six reactivators with which this report is concerned. For example, complaint of a bitter, metallic, salty or musty taste has been made by people who have been given any of the six oximes, whether by mouth or by injection. Intravenous injections of any of the oximes, if the solutions were too concentrated or were given too rapidly, have resulted in pain along the vein. Dizziness, nausea possibly progressing to vomiting, blurred vision with impaired accommodation, and muscular weakness have been complained about often. Convulsions have been reported after V.[59,63] Moderately marked increases in systolic and diastolic blood pressures with increased pulse pressures and tachycardia have been reported to follow intravenous administration of 2-PAM I, I, and II.[59,63,69,72,73] The increases after II were not as great as those after I. The two bisquaternary bis-oximes (III and IV) may produce initial increases in blood pressure, but these are followed by marked and prolonged hypotension, the pulse pressure being reduced progressively after administration of the oxime.[72,77] Obidoxime given by mouth did not alter blood pressure.[81]

Another symptom of some practical importance is gastrointestinal distress, evident especially when oximes were given repetitively during several days. This symptom has been particularly bothersome with II and III,[72,80] but has been reported after large doses of I also.[76] III and IV have induced symptoms that suggested local anesthetic effects: sensations of heat or coolness in the nose and throat, circumoral numbness, and facial paresthesia.[72,73,77,80] III has given rise to icterus, petechial bleeding with increases in prothrombin time and sedimentation rate, increases in serum alkaline phosphatase, glutamic-oxaloacetic transaminase, and glutamic-pyruvic transaminase activities, an increase in serum bilirubin concentration, and a fine macular rash on the face and arms.[72] IV may have resulted in cholestatic hepatosis,[73] but 11 persons (six men and five women) given two intramuscular injections of 250 mg of IV 2 h apart on one day had no significant increases in their serum alkaline phosphatase, glutamic-oxaloacetic transaminase, glutamic-pyruvic transaminase, glutamate dehydrogenase, or sorbitol dehydrogenase activities thereafter.[78] Also, four oral doses making a total of 7.36 g of IV on one day induced no significant alterations in serum glutamic-oxaloacetic and glutamic-pyruvic transaminase activities in 10 people who were given that dose, although four of them complained of some side effects.[81] Facial paresthesia and headache were the principal complaints of the 13 subjects, in a group of 24, who mentioned symptoms after oral doses of 1.84-7.36 g of IV.

Sidell et al.[80] reported that intramuscular injection of I or sodium chloride into normal human subjects resulted in increases in serum creatine phosphokinase activity. When the concentrations of the solutes in the solutions were expressed as milliosmols per liter, sodium chloride and I yielded nearly parallel relationships between the increase in serum creatine phosphokinase activity and the amount of solute administered, I being 2.4-3.5 times as active as sodium chloride in inducing an increase in activity. The data furnished by these investigators indicate that, when the volume of solution injected into muscle was constant, the release of creatine phosphokinase from the muscle was related directly to the osmolarity of the solution; when the osmolarity was kept constant, the amount of creatine phosphokinase released was a function of the amount of material injected into the muscle. A graph of the increase in serum creatine phosphokinase activity as a function of the amount of I injected extrapolates to a point close to the origin, whereas the line of the increase in serum creatine phosphokinase activity as a function of the concentration of sodium chloride injected extrapolates to zero at a concentration of 4%, indicating that I had an effect on the integrity of cellular membranes beyond that due simply to osmotic relationships. The report by Wedd and Burgess[61] that intramuscular injection of a 25% solution of II produced no more damage than intramuscular injection of a 7.5% solution of sodium chloride suggests that II may not have the extra damaging effect on cellular membranes that I seems to have. In this regard, consideration should be given to the possibility that the biochemical indicator of effect used by Sidell et al. may be more sensitive than the histologic indicator used by Wedd and Burgess.

Obidoxime has been stated[81] not to have any local irritant action when injected intramuscularly; it did produce facial paresthesia, headache, a sensation of coolness in the mouth, generalized weakness, nausea and vomiting, pallor, pyrosis, and sore throat in 13 of 24 subjects given tablets of IV in doses of 1.84-7.36 g. Administration of IV in tablets with enteric coatings probably did not alter the incidence of side reactions--only four subjects and two doses of the oxime were used in this part of the study.

MODIFICATIONS OF NORMAL FUNCTIONS BY OXIMES

The oximes were developed originally as reactivators of inhibited cholinesterase by evolution from hydroxylamine through hydroxamic acids to oximes. Kinetic studies with hydroxamic acids indicated that the hydroxamate ions were the reactivating agents.[82] On the assumption that the anionic site in inhibited cholinesterase is still operational, so that a positively charged site in a reactivator could be used as a directing group to guide the active group in a molecule of a reactivator to the inhibited esteratic site of the enzyme, compounds containing quaternized amino groups were made. This chemical change in, for example, pyridine-2-aldoxime also lowered the pK of the resulting pyridinium oxime and increases its ability to move into tissues. Methyl nicotinium hydroxamic acid iodide and later 2-PAM I--both having a quaternized nitrogen atom removed by about two carbon atoms from a hydroxylated nitrogen atom--were found to be effective reactivators of cholinesterase inactivated by organophosphorus inhibitors.[2,4,82] Although the truth of the basic assumption was not demonstrated until 1959,[84] the general success of quaternization in increasing the reactivating potencies of hydroxamic acids and oximes with pyridine skeletons made it almost certain before then. Oximes were found to be much more active than the corresponding hydroxamic acids.[83]

At about the same time, hydroxamic acids and oximes were found to react directly with organophosphorus compounds.[84,85] 2-PAM I was found to react in vitro with sarin with marked deviation from first-order kinetics; that suggested that the reaction actually consists of (at least) two sequential reactions. Green[86] showed that quaternized pyridine aldoximes react with an organophosphorus (OP) compound in three steps:

- Formation of a phosphorylated or phosphonylated oxime by reaction of the OP compound with the oximate ion.

- Hydroxylation of the phosphorylated or phosphonylated oxime to produce an *N*-alkylcyanopyridinium salt.

- Hydrolysis of the *N*-alkylcyanopyridinium compound to an *N*-alkylpyridone and hydrogen cyanide.

The second-order rate constant for the reaction between sarin and either 2-PAM I or II was found to be 170 L/mol per minute. If a phosphorylated or phosphonylated oxime that does not enter rapidly into the second step above is formed, that product may be an inhibitor of cholinesterase.[87,88] Hydrolysis of sarin in the presence of 200-fold concentrations of V and II took place more rapidly in plasma from rats with the former oxime than with the latter.[89] In human plasma, sarin was hydrolyzed more rapidly in the presence of II than in that of V.

The protective activity of IV against lethal intoxication by OP compounds has been thought to be exerted principally by promoting reactivation of inhibited cholinesterase.[90] IV has been found to produce either activation or inhibition of acetylcholinesterase, depending on the simultaneous concentrations of that oxime and of acetylcholine in the vicinity of the enzyme.[91] With acetylcholine at 5×10^{-4} M, concentrations of IV below about 3×10^{-4} M produced activation of cholinesterase; greater concentrations of the oxime inhibited cholinesterase. Increasing the concentration of acetylcholine to 10^{-2} M decreased progressively both the activating and inhibitory actions of IV. With the substrate at 10^{-2} M, the activity of the enzyme was not altered appreciably by IV at 5×10^{-6} M to 7×10^{-3} M. These findings suggest that acetylcholine and IV react with different, but interdependent, receptors on the molecule of acetylcholinesterase; the data contain no evidence of competition between IV and acetylcholine for a common receptor.

Holmes and Robins[45] reported that 2-PAM I overcame neuromuscular blockade induced by DFP, TEPP, or sarin, but this could be demonstrated with the isolated phrenic nerve-diaphragm preparation from the rat only after washing away excess OP compound. Intravenous injection of 2-PAM I overcame slowly neuromuscular blockade induced by OP compounds. The oxime, they found, also had a direct toxic action on muscle, reducing the ability of muscle to shorten and decreasing the ability of muscle fibers to conduct impulses. The same investigators reported[92] that V (intraperitoneally at 150 mg/kg) had little, if any, effect on a sarin-induced blockade of neuromuscular transmission.

Wills and Kunkel[93] studied a group of 46 compounds--d-tubocurarine chloride, decamethonium bromide, gallamine triethiodide, a series of quaternized bisoxamide salts, a group of tertiary and quaternary anticholinergic compounds, and a group of hydroxamic acids and oximes--for their ability to antagonize a sarin-induced blockade of neuromuscular transmission to the cat's gastrocnemius-soleus muscle group during indirect stimulation at a frequency of 1 shock every 2 s. They identified d-tubocurarine chloride, two of the oxamide derivatives, and a quaternary anticholinergic and antihistaminic compound as particularly active antagonists of sarin's effect. 2-PAM I and 2-PAM benzyl bromide had comparatively low activity. V had only slight, if any, activity in this test. When the frequency of indirect stimulation was raised to 40/s, 2-PAM I became much more effective in antagonizing blockade of neuromuscular transmission than an atropinium salt that had had about the same activity as 2-PAM I against blockade at the lower frequency of stimulation.

Fleisher[94] reported that both sarin and VX increased the sensitivity of the isolated frog's rectus abdominis to external application of acetylcholine and at the same time decreased the activity of cholinesterase in the external surfaces of the muscle cells. Sarin at 5×10^{-7} M reduced the threshold concentration of acetylcholine for inducing contraction of the muscle to 8% of that required before application of sarin. The same concentration of VX reduced the threshold concentration of acetylcholine to 6.7% of that needed previously. Contemporaneously, the activity of cholinesterase in the external surfaces of the muscle cells was reduced to 8.1% and 0% of that before application of sarin and VX, respectively. Addition of 2-PAM I at 5×10^{-4} M to the baths in which the muscles were suspended had little effect on the activity of the enzyme in homogenates of the muscles, but restored 75% and 91%, respectively, of the activity of cholinesterase in the external surfaces of the muscles exposed to sarin and VX. At the same time, the concentration of acetylcholine required to induce contraction of the muscles was raised to 53.3% and 58.3% of the original threshold concentrations, respectively, for the muscles exposed to sarin and to VX.

Rats poisoned by subcutaneous injection of VX at twice the LD_{50} were kept alive for 20 min with artificial ventilation of the lungs when necessary.[95] At 20 min after the dose of VX, some rats were given intraperitoneal injections of either atropine sulfate (7 mg/kg) or atropine sulfate plus 2-PAM I (17.5 mg/kg). Untreated rats were killed 20 min after injection of VX; samples of parotid gland, gastrocnemius muscle, and brain were collected for examination for cholinesterase activity. The treated rats were killed 3 h after treatment. Organ samples were collected and analyzed for cholinesterase activity. Reactivation of cholinesterase was calculated as 100 times the ratio of the difference between cholinesterase activities 3 h after therapy and 20 min after VX to the difference between cholinesterase activities in unpoisoned rats and in poisoned rats 20 min after VX; it is shown in Table 3 for the two modes of therapy. Because atropine has never been found to have reactivating activity _in vitro_, the reactivation that occurred in the rats treated with atropine sulfate is assumed to be spontaneous. It is apparent from Table 3 that addition of 2-PAM I to atropine increased cholinesterase reactivation by 40.5% in the parotid gland, by 127.8% in the gastrocnemius muscle, and by 8.2% in the brain. The especially large change in cholinesterase activity in skeletal muscle suggests that this may be the principal site at which 2-PAM I antagonizes inhibition of cholinesterase.

The finding by Fleisher _et al._[47] that 2-PAM I facilitated response by skeletal muscle to acetylcholine and depressed responses to decamethonium and carbamylcholine, whereas III had only the depressant actions, suggests that 2-PAM has an activity that is not duplicated in III. The nature of this difference is not entirely clear.

TABLE 3

Recovery of Cholinesterase Activity in Parotid Glands, Gastrocnemius Muscles, and Brains of Rats Poisoned with Subcutaneous VX at 40 μg/kg and Treated 20 Min Later with Intraperitoneal Atropine Sulfate at 7 mg/kg or with Intraperitoneal Atropine Sulfate and 2-PAM I at 17.5 mg/kg[a]

	Reactivation, % of inhibition by VX		
Therapy	Parotid Gland	Gastrocnemius Muscle	Brain
Atropine sulfate	39.0	25.2	8.5
Atropine sulfate + 2-PAM I	54.8	57.4	9.2

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[a] Samples of tissue were removed 3 h after therapy, or 20 min after VX if no therapy was administered. Groups of rats contained a mean of 8 (6-15) animals each.

Because 2-PAM adds to the depressant effect of d-tubocurarine on the response of skeletal muscle to indirect stimulation, as well as to those of decamethonium and carbamylcholine, the facilitating action cannot be based on depolarizing activity. Fleisher et al. suggested that the facilitating component of the action of salts of 2-PAM depends on the ability of this oxime to inhibit cholinesterase, rather than on a direct depolarizing action. The finding that salts of 2-PAM depress the response of eserinized muscle to acetylcholine and to the inhibiting action of d-tubocurarine points to the possession by 2-PAM of not only a cholinesterase-inhibiting action but also a competitive interference with acetylcholine uptake by the motor endplate analogous to that of d-tubocurarine itself. The accentuation by salts of 2-PAM of blockade of neuromuscular transmission by such depolarizing compounds as decamethonium and carbamylcholine would be analogous to that of d-tubocurarine in increasing Phase II blockade by depolarizing compounds. Although III is a more potent inhibitor of acetylcholinesterase in vitro than the salts of 2-PAM, its effects on muscle seem to be those of a competitor with acetylcholine for access to the motor endplate.

Edery[96] studied the effects of V on neuromuscular blockade induced by ethyl pyrophosphate or neostigmine methylsulfate in the cat, finding only an insignificant effect. The skeletal muscle responses to both direct and indirect excitation were altered by V in the absence of any other active chemical. The effect of V on the response of indirectly stimulated muscle to d-tubocurarine was an increase in the blockade of neuromuscular transmission similar to that of the salts of 2-PAM and of III.

Table 3 shows that cholinesterase inhibition in brain is affected only slightly by a dose of 2-PAM I that produces significant reactivation of the enyzme in muscle and parotid gland. Indeed, there has been considerable controversy about the ability of oximes to enter the CNS and reactivate inhibited cholinesterase there. Several clinical observations suggest that comparatively small doses of oximes have significant effects on cerebral function. For example, Schuchter et al.[97] reported a case of poisoning by parathion treated initially with atropine alone. After administration of 30.5 mg of atropine sulfate during the 14 h after admission to hospital, the apnea, convulsions, and arrhythmic tachycardia that had characterized the patient's condition on admission had stopped. The patient was still unconscious, however, with markedly constricted pupils. At that time, intravenous injection of 0.5 g of 2-PAM I in a 1% solution relieved both residual effects immediately, and no further treatment was required. Several similar reports are available in the medical literature; they suggest that, even though the quaternized oximes may not be able to cross the blood-brain barrier in large amounts, either the small quantities that penetrate that barrier are capable of affecting the function of crucial parts of the brain or the oximes affect the functions of peripheral structures (perhaps receptors of sensory input) that modify the functions of crucial parts of the brain. The latter possibility is related to the proposal of Erdmann *et al.*[98] that restoration by 2-PAM I of the righting reflex after its abolition by parathion is effected by the oxime's modifying inhibition of cholinesterase in some peripheral site important for activity of the reflex.

Rajapurkar and Koelle[99] reported that intravenous V at 40 mg/kg, but not at 4 mg/kg, induced reactivation of cholinesterase in the surfaces of cells of the cat's superior cervical ganglion after the animal had been given DFP at 3.7 mg/kg 20 min earlier; there was no significant reactivation of the cholinesterase in the ganglion as a whole (after homogenization). These findings suggest that, even when an oxime is able to make contact with the surfaces of nerve cells, it is not able to penetrate into the neurons; this is similar to the situation for muscle cells described by Fleisher.[94].

Schaumann[100] found that pretreatment of mice with 2-PAM I reduced inhibition of acetylcholinesterase in brain by paraoxon much more effectively than those by DFP and 217-AO. The finding of some protection against all three OP compounds could depend on direct reaction between the last two inhibitors and the oxime, with a reduction in inhibition of the enzyme. A similar consideration applies to the report by Bisa *et al.*[101] that IV protected serum and brain cholinesterase from inhibition by paraoxon administered later at twice the LD_{50}. Although the same intraperitoneal dose of IV (7 mg) was found to protect the cholinesterase of rat serum and brain only incompletely from inhibition by DFP at 5 times the LD_{50}, that of serum recovered its normal activity by 20 h after the dose of DFP, whereas that of brain required 26 h for recovery.

On comparison of cholinesterase activities in the brain on various days after gavage with parathion at 35 mg/kg and after exposure of a homogenate of brain *in vitro* to 10^{-3} M 2-PAM I, the reactivation accomplished by the oxime was found[100] to decrease progressively as spontaneous reactivation increased:

Day	Brain Cholinesterase, % of normal Before 2-PAM I	After 2-PAM I
0	6	100
1	20	59
4	44	79
8	63	76

In vitro, addition of IV and DFP simultaneously to red-cell cholinesterase[90] protected the enzyme against inhibition by DFP to a greater extent than the reactivation that the same concentration of the oxime (10^{-3} M) was capable of effecting after exposure of the enzyme to the same concentrations of DFP ($1\text{-}3 \times 10^{-7}$ g/ml). In guinea pigs, simultaneous injections of IV (intramuscularly at 100 mg/kg) and DFP (subcutaneously at 1.5 mg/kg) limited inhibition of red-cell cholinesterase to 20%, whereas the same dose of DFP alone inhibited it by about 65%. Half that dose of IV injected intramuscularly 90 min after the same dose of DFP stopped inhibition of red-cell cholinesterase by DFP and returned the activity of that enzyme to 93% of its normal value by 24 h after the dose of DFP. The red-cell cholinesterase of guinea pigs that were not given oxime after the dose of DFP had risen to only 87% of its normal value by the twenty-first day after injection of DFP.

In dogs poisoned with soman (intravenously at 30 μg/kg) and treated with I at 104 mg/kg (intravenously 3 1/2 min after soman), the large dose of I stopped aging of inhibited cholinesterase and reactivated 24.0% and 35.6% of the red-cell and diaphragm cholinesterase activities, respectively. It failed to reactivate brain cholinesterase. Indeed, the brain acetylcholinesterase activity after the treatment with I was lower than that just before the injection of I. The last finding indicates the inability of I to cross the blood-brain barrier in significant quantities.

Apparently on the other side of the picture is a report by Meeter[104] that intraperitoneal injection of either III or IV at 40 mg/kg, given to rats when their body temperatures had fallen 2-2.5°C after intravenous injections of DFP at 1.2 mg/kg, blunted the fall, which might proceed in untreated rats to a decrease of nearly 6 °C. These doses also shortened the return of body temperature to its normal value. Larger doses of the same oximes blunted the fall less than the smaller dose, but had more effect on the rate of recovery of body temperature to its normal value. From the fact that atropine also is a good antagonist of the hypothermic action of OP compounds, whereas an equivalent dose of methyl atropinium nitrate has essentially no antagonistic activity, the hypothermia seems to result from some effect of an OP compound in the CNS. On this basis, one supposes that the moderate doses of III and IV antagonize some effect of OP compounds on the CNS that leads to hypothermia and that larger doses of the same oximes exert initially a toxic action that may actually increase hypothermia. As the concentration of the oxime in the body falls back toward that established by the modest dose, the antagonistic activity becomes evident. It would be useful to know whether oximes are able to increase the partial antagonism of inception of hypothermia that atropine has been found to exert.

A dose of IV given intramuscularly to atropinized guinea pigs 0.5 h after a sublethal dose of sarin produced less reactivation of retinal and brain cholinesterase than an equimolar dose of I.[105] A reactivation of 23.9% of the inhibited cholinesterase in the retina by I was accompanied by one of only 3.7% in the brain. If IV produces less reactivation of inhibited cholinesterase in the brain than I, any central mechanism related to the induction of hypothermia by OP compounds must be either very sensitive to the concentration of acetylcholine within the brain or more permeable to the quaternized oximes than the brain in general.

Atropine has been found[106] to reduce markedly the increase in the concentration of total brain acetylcholine in rats later given paraoxon at 0.4 mg/kg. Rats given a dose of paraoxon and than treated with IV intraperitoneally had brain concentrations of free and total acetylcholine that were essentially the same as those in rats given paraoxon alone, but no tremors or convulsions were observed. These animals survived; those given paraoxon alone all died in convulsions within 3-8 min.

Bajgar _et al._[107] found that acetylcholinesterase activity in the pontomedullary area of the mouse brain was possibly a direct linear function of the dose of IV (2.5-35.0 mg/kg) injected intramuscularly with a constant dose (21 mg/kg) of atropine sulfate 30 s after an intramuscular dose (400 μg/kg) of sarin. They found no significant relation between the dose of IV and acetylcholinesterase activity in mesencephalon, diencephalon, and basal ganglia in the same experiments. Mortalities in the groups of mice given various doses of IV seemed to be inversely related to the dose of the oxime and, therefore, to cholinesterase activity in the pontomedullary region. Vasić _et al._,[108] using armin as the OP compound, found that IV (intraperitoneally at 25 mg/kg 5 min after subcutaneous injection of armin at 0.4 mg/kg) resulted in a cholinesterase activity in the pontomedullary region of the rat's brain 45.3% of that in control animals, whereas this region in rats given armin alone contained only 18.7% of the cholinesterase activity of controls. The decrease in cholinesterase activity was accompanied by increases in the total acetylcholine concentration in the pontomedullary region of 52% in the rats given armin alone and of 25% in those given both armin and IV.

2-PAM I seems to be less effective than IV in reactivating brain cholinesterase after its inactivation by paraoxon or other OP compounds.[109] There was early evidence that 2-PAM produced more reactivation of cholinesterase in the pontomedullary region and the area postrema that had been inhibited by paraoxon than in the cerebellum and the cerebral cortex[110] and that it could prevent the appearance of grand mal-like discharges in EEGs of rabbits after doses of sarin that evoked such discharges in rabbits not protected with 2-PAM I.[111] This report by Longo et al. is not completely satisfying in indicating penetration of a quaternary oxime into the brain, inasmuch as the protective effect could arise from direct reaction between the oxime and sarin in the blood; in that case, only a part of the sarin administered would be available to affect cholinesterase in the brain and other tissues. The protective effect of the dose of 2-PAM I (30-50 mg intravenously in rabbits weighing 2-3 kg) was found to be overcome by doubling or tripling the dose of sarin. This finding suggests that the protective action of the 2-PAM I was due primarily to direct reaction with sarin.

In the final study to be mentioned in regard to the possible penetration of pyridinium oximes into brain, anesthetized, atropinized cats were given intravenous injections of sarin at 27 μg/kg.[112] Thirty minutes later, to allow clearance of unreacted sarin from the tissues, some cats received saline injections into one common carotid artery and others received similar injections of I at 15 mg/kg. The cholinesterase activity of cerebral cortex was measured. In animals given I, nearly 20% of the cholinesterase in their cerebral cortices that had been inhibited by sarin was calculated to have been reactivated by the oxime.

The only functional changes noticed in cats and dogs (anesthetized with sodium pentobarbital) after intravenous 2-PAM I at 5-60 mg/kg were stimulation of respiration (principally increased depth) and an increase in pulse pressure.[113] The mean blood pressure, after a small dip immediately after the injection, returned approximately to the control value. With repeated doses of 5 and 10 mg/kg, the heart rate decreased after each dose and then returned gradually to its normal value. After a cumulative dose of 100 mg/kg, there was marked lowering of the heart rate and a decrease in the voltage of the T-wave of the ECG. Isolated hearts from rabbits perfused with 2-PAM I at 1.3-2.7 g/L in the Ringer-Locke solution underwent no alteration of normal activity other than a slight decrease in the rate of beating, even when the perfusion was continued for more than an hour. In cats, intravenous injection of 2-PAM I at 40 mg/kg or more markedly increased the peristaltic activity of the intestines. Atropine stopped this effect.

Supramaximal indirect stimulation at 30/min of the cat's gastrocnemius-soleus muscle group was blocked by close intra-arterial injection of 2-PAM I at 80 mg/kg. By the intravenous route, the oxime at 80-100 mg/kg induced a persistent increase in the tension developed during a twitch. An intravenous dose of 300 mg/kg resulted in a brief increase in the tension developed during a twitch followed by a decrease in the tension lasting for about 2 min. This dose resulted also in a sequence of changes in blood pressure: brief hypertension, hypotension with increasing pulse pressure, and slight hypertension with greatly increased pulse pressure. Respiration, recorded from the pressure in the side arm of a T-shaped tracheal cannula, went through a sequence of changes similar to those in blood pressure: a brief increase in depth of respiration, a brief decrease in depth of respiration, increasing depth of respiration with an increased rate, and greatly increased depth of respiration at a rate somewhat below the original rate. Mydriasis and muscular fasciculation were observed also after this large dose of 2-PAM I.

Blockade of neuromuscular transmission produced by an intravenous dose of d-tubocurarine chloride at 0.3 mg/kg, but not that due to 0.5 mg/kg, was antagonized by intravenous 2-PAM I at 5 mg/kg. Edrophonium bromide (intravenously at 0.2 mg/kg) was a more potent antagonist of d-tubocurarine than this dose of 2-PAM I. Partial blockades of neuromuscular transmission induced by intravenous injections of decamethonium bromide at 20 μg/kg, neostigmine bromide at 1 mg/kg, or succinylcholine chloride at 50 μg/kg were intensified by intravenous injection of 2-PAM I at 5 mg/kg, in about the same way in which they were enhanced by intravenous doses of edrophonium bromide at 0.2 mg/kg. Intravenous doses of 2-PAM I at up to 150 mg/kg had no effect on the muscle response to direct stimulation in anesthetized cats.

Doses of 2-PAM I larger than 40 mg/kg produced brief blockade of the cardiac response to peripheral vagal stimulation and of the nictitating membrane's response to preganglionic, but not to postganglionic, stimulation of the cervical sympathetic trunk. Reactions in which postganglionic adrenergic effectors participate--such as mydriasis, pressor response to injected acetylcholine by atropinized animals, pressor response to injected epinephrine, and pressor response to bilateral occlusion of the common carotid arteries--were augmented definitely by intravenous injection of 2-PAM I at 20 mg/kg or more. No significant effect of 2-PAM I on the EEGs of curarized cats was recorded.

In summary, 2-PAM 1 was found by Kunkel et al.[113] to act as a depolarizing compound at the neuromyal junction and to have acetylcholinomimetic properties in large doses. It had some ganglionic blocking activity, but no direct effect on the CNS was detected.

A later paper presented the results of a study of the mechanism of the sympathomimetic cardiovascular actions of I.[114] Cats and dogs anesthetized with allobarbital-urethane were used for measurements of pressure near the bifurcation of the descending aorta and of blood flow with electromagnetic probes. Intravenous injection of I at 20 mg/kg was found to produce an immediate, sharp increase in blood pressure lasting for about 25 s and followed after a lag of about 6 s by an increase in blood flow. A slow drift downward of the peak systolic pressure followed. Repetition of the dose of I after an hour yielded responses similar to those after the first dose, but with a somewhat larger and more persistent immediate effect on blood flow. Calculation of the peripheral resistance and graphing of simultaneous plots of peripheral resistance, peak systolic blood pressure, blood flow, and contractile force of the heart (from Walton strain-gauge arches attached to the outer surface of the ventricles) revealed that the peak systolic blood pressure paralleled more faithfully the peripheral resistance than it did any of the other variables and that the peripheral resistance increased more abruptly after a third dose of I at 30 mg/kg than after the second such dose, which in turn induced a larger increase than the first dose. The blood flow decreased to 55% of the original value during the 76 min between the peak flow after the second dose of I and the third dose. It increased only slightly after the third dose.

A dose of I at 30 mg/kg increased the effects of intravenous doses of epinephrine at 5 g/kg and of dl-norepinephrine at 10 μg/kg on both blood flow and blood pressure. Intravenous phenoxybenzamine at 15 mg/kg plus tolazoline at 2 mg/kg prevented almost completely the actions of I on blood pressure and blood flow. Intravenous reserpine at 2 mg/kg increased markedly the effects of I at 30 mg/kg on blood pressure and peripheral resistance, but converted the usual immediate, small, temporary increase in blood flow into an immediate, small, temporary decrease. These various responses would be expected from either a mild sympathomimetic amine or an inhibitor of the breakdown of endogenous catecholamines. Indeed, I at 10^{-4} M, was found to inhibit the monoamineoxidase of the rat's liver. If the dose of I used in these experiments were distributed into the same fraction of the body water as that estimated for the human body,[115] the concentration in the plasma would be about 9 times that stated above as the effective concentration for inhibiting the monoamineoxidase. It is possible that inhibition of monoamineoxidase by I plays a part in inducing the effects of the oxime on blood vessels and blood pressure. It is possible also that I interferes with reuptake of catecholamines by nerve endings; this possibility seems not to have been explored.

Another study of the effects of I on the cardiovascular system[116] concluded that, in dogs anesthetized with sodium pentobarbital, the response of blood pressure to intravenous administration of I is a resultant of two separate effects: a direct myocardial stimulation that was stopped with dichloroisoproterenol and a stimulation of vascular smooth muscle that results in a slight increase in renal arterial pressure and a slight decrease in renal arterial flow. Neither atropine nor dichloroisoproterenol affected these vascular effects. Injections of I into a jugular vein or a renal artery had no consistent effect on catecholamine concentrations in plasma taken from a femoral artery or a renal vein. In seven experiments in which I at 21-35 mg/kg was injected into a jugular vein, the mean blood pressure increased from 176/125 ± 22/11 to 186/124 ± 53/39 mm Hg, and the mean concentration of catecholamines in the plasma of femoral arterial blood went from 1.7 ± 0.7 to 1.9 ± 1.3 μg/L. In three experiments in which 100 mg of I was injected into the renal arteries of canine kidneys that weighed a mean of 62.3 g (body weights not stated), renal blood flow changed from a mean of 233 ± 2.9 ml/min to 227 ± 8.2 ml/min. Mean catecholamine concentrations in renal arterial blood changed from 1.5 ± 0.3 to 2.1 ± 0.4 g/L of plasma, and those in renal vein blood from 1.5 ± 0.5 to 2.0 ± 0.3 μg/L.

Another study of the hypertensive action of I was made by DiPalma and associates.[117] Dogs anesthetized with allobarbital-urethane and cats anesthetized with urethane or by section of the cervical spinal cord during anesthesia with ether were used. In 11 dogs, intravenous injection of I at 20 mg/kg induced an increase in mean blood pressure from 160/103 ± 28/22 to 201/130 ± 28/23 mm Hg at 2 min after injection; by 20 min after injection, the mean blood pressure was 185/119 ± 35/27 mm Hg. Phenoxybenzamine (intravenously at 1 mg/kg) and phentolamine (intravenously at 1 mg/kg) consistently blocked the hypertensive action of I when they were injected a few minutes before I. Hexamethonium (intravenously at 1-2 mg/kg 10 min before I) did not prevent the increase in blood pressure, but did eliminate the initial period of bradycardia and the initial spike of hypertension; slow development of an increase in blood pressure still occurred. Pentolinium tartrate (at 2 mg/kg), succinylcholine chloride (at 1 mg/kg), guanethidine (at 1-3 mg/kg), and d-tubocurarine chloride (at 1.2 mg/kg) were ineffective in preventing the increase in blood pressure.

Dogs given norepinephrine by infusion at 0.185-2.04 μg/kg per minute for long periods (9-35 h) until their blood pressures became approximately the same as they had been before the infusions began were then given intravenous injections of I at 20 or 40 mg/kg, tyramine at 0.1 or 0.5 mg/kg, or nicotine at 0.5 mg/kg.[118] The concentration of norepinephrine in carotid arterial blood was estimated before and at intervals after the injection of one of the test compounds. Except after the lower dose of tyramine, when the maximal increase in the concentration of norepinephrine in the blood appeared at 5 min after the injection, the peak concentration of norepinephrine in carotid arterial blood occurred at 3 min after injection. The release of norepinephrine induced by I at 40 mg/kg was about 1.35 times that produced by the lower dose. The release of norepinephrine induced by tyramine at 0.5 mg/kg was 1.47 times that by I at 40 mg/kg. The release by nicotine at 0.5 mg/kg was 1.59 times that by the larger dose of I. The action of I in inducing release of norepinephrine from tissues in which it has been stored, although it is slightly more prolonged (20 min vs. 15 min) than those of the classical releasers of norepinephrine, is much weaker than those of tyramine and nicotine.

Still, release of catecholamines may play a part in the genesis of hypertension after injection of a pyridinium oxime.

Dogs anesthetized with sodium pentobarbital were used in experiments in which the lungs were mechanically ventilated after the dogs' chests had been opened to place electromagnetic flowmeters around the ascending aorta and catheters into the left atrial appendage.[119] Dose-response curves for doses of I at 5-40 mg/kg in the absence and presence of blockade of β-adrenergic receptors (phentolamine), of blockade of β-adrenergic receptors (propranolol), or of depletion of catecholamines (reserpine) were constructed. The heart rate was decreased progressively by intravenous I at 5 and 10 mg/kg; larger doses of 20 and 40 mg/kg yielded serially smaller effects on heart rate. Phentolamine (1 mg/kg) increased the effects of the two lower doses of I on heart rate, but antagonized those of the two larger doses, converting the usual slight bradycardiac effect of 40 mg/kg into a mild tachycardiac one. Both propranolol (0.5 mg/kg) and reserpine (0.5 mg/kg during 3 d) tended to increase the bradycardiac effect of all doses of I. The increase in peripheral resistance induced by I reached significance only after the largest two doses; this effect was antagonized by phentolamine and was increased by both propranolol and reserpine. The stroke volume of the heart was increased progressively by the four doses of I used; this effect was antagonized to some extent by all three drugs and especially by the two blockers of adrenergic receptors. Cardiac output was increased progressively by increasing doses of I and also was antagonized to some extent by all three drugs. Propranolol and reserpine had especially marked antagonistic actions. Arterial pressure, which is a resultant of cardiac output and peripheral resistance, was increased progressively by the four doses of I. The hypertensive action was antagonized especially by reserpine and phentolamine, propranolol having only a minor antagonistic action, according to Barnes et al.[119]

Taken together with the results obtained by DiPalma's group,[117,118] the finding by Barnes et al.[119] of the particularly large effect of prior treatment with reserpine on the response of the arterial pressure to I seems to confirm the involvement of catecholamine release in this hypertensive response. It is clear, however, that I also has a direct inotropic effect on the heart, in that the increase in stroke volume was blocked only partially by any of the three possible antagonists used by Barnes et al. In view of the fact that none of the possible antagonists was able to prevent more than about 68.5% of the increase in peripheral resistance induced by I, this oxime may well have direct stimulant effects on vascular smooth muscle.

III, unlike I, was found[49] to lower blood pressure and produce bradycardia in cats anesthetized with sodium pentobarbital. Partial heart block and inversion of the T wave of the ECG followed the administration of large doses of this oxime. Moderate doses (5 and 10 mg/kg) produced no noticeable changes in peristaltic or respiratory activities; III at 20 mg/kg induced temporary decreases in both these activities. Doses of 40-80 mg/kg produced respiratory failure and spastic contraction of intestinal muscles. Neuromuscular transmission of either single or repetitive stimuli was not affected by doses of 5 or 10 mg/kg. A dose of 20 mg/kg resulted in a marked, temporary decrease in the tension developed during indirect tetanic stimulation, but was without effect on the twitch response of the gastrocnemius-soleus muscle group. Doses to and including 40 mg/kg did not alter responses to bilateral occlusion of the carotid arteries and to intravenous injections of acetylcholine or epinephrine at 3 μg/kg; 40 mg/kg partially blocked the cardiovascular response to stimulation of the peripheral vagi. This oxime was found to be an effective adjunct to atropine in both prophylaxis and treatment of intoxication by sarin and VX in anesthetized cats and dogs.

Bay[120] made a more extensive study of the efects of III on blood pressure, respiration, and ganglionic and neuromuscular transmission. Intravenous doses of 25 mg/kg or more decreased systolic and diastolic pressure in anesthetized cats and dogs. In the cat, a dose of 30 mg/kg slightly increased the pressor effect of epinephrine at 5 μg/kg and slightly decreased the depressor effect of acetylcholine at 3 μg/kg. III doses of 2.5-40 mg/kg were found to yield linear log dose-response curves for inhibition of effects induced by preganglionic stimulation of sympathetic and parasympathetic ganglia; the effect on the response to stimulation of preganglionic neurons by sympathetic ganglia was 1.4-2.2 times that on the response to similar stimulation by parasympathetic ganglia (the larger factor applies to the lower doses of the oxime). The transient hypotension was attributed largely to the action of III in blocking ganglionic transmission and partially to a direct relaxant action on vascular smooth muscle. Moderate doses of III temporarily increased the breathing rate and transiently decreased the tension developed during an indirectly stimulated twitch response by the gastrocnemius muscle. Doses of III greater than 50 mg/kg abolished the indirectly stimulated twitch response of the muscle and reduced its ability to maintain a tetanic response to repetitive indirect stimulation. The response of skeletal muscle to direct stimulation was not altered. Neostigmine, edrophonium, and K^+ ions antagonized, whereas d-tubocurarine increased, the effects of III on the neuromyal junction, which seemed to arise from competition with acetylcholine for access to the receptor.

In cats anesthetized with sodium pentobarbital, an intravenous dose of III at 25 mg/kg was found to be able to block almost comletely the cardiac response to stimulation of the peripheral right vagus nerve.[121] Small doses (2-8 mg/kg) did not inhibit peristaltic activity; in fact, an increase in the rate of contractions was seen. II and V had effects on response by the heart to stimulation of the vagus that were similar quantitatively to that of III, but were weaker. Intestinal contraction induced by vagal stimulation was blocked by III, but not by twice as large a dose of V. Lindgren and Sundwall[121] found that the response of the nictitating membrane to stimulation of the cervical sympathetic chain was almost unaffected by III; thus, they differed with Bay on the relative sympatholytic and parasympatholytic activities of III. These investigators concluded that III probably exerts its vagolytic action by competition with acetylcholine.

Wellhöner _et al._[122] reported that intravenous injection of III at 25 mg/kg led to marked hypotension in rats, guinea pigs, rabbits, and cats. The hypotensive effect was particularly marked in old cats that had high blood pressures initially; it was not altered by bilateral vagotomy, evisceration, or removal of the carotid bodies, but was reduced or abolished by decapitation. These investigators suggested that the hypotension may result from some effect of the oxime on the CNS. They found also that prior doses of III prevented the hypertensive action of norepinephrine.

Kunkel _et al._[50] found that both I and III at 15 mg/kg blocked the response of the heart to stimulation of the vagus nerve. Although I increased the depressor action of acetylcholine, III did not alter it. I increased the effects of injected epinephrine on blood pressure and contraction of the nictitating membrane, but decreased the response of the nictitating membrane to stimulation of the cervical sympathetic trunk. These findings fairly well localized the blocking action of this oxime to the ganglion itself. In contrast, III had no effect on the pressor action of injected epinephrine, partially blocked stimulation of the nictitating membrane by injected epinephrine, and blocked the response of the nictitating membrane to preganglionic stimulation of the superior cervical ganglion. On the basis of these observations, one would think that III affected the receptors in both the ganglion and the nictitating membrane. The pressor effect of acetylcholine in atropinized dogs was increased by I, but not by III. III decreased the pressor effect of bilateral occlusion of the carotid arteries in cats, whereas I did not. It is possible, therefore, that III affects the inferior petrosal ganglion, which includes the somas of the pressoreceptors in the carotid sinus, or the reticular formation of the pons and medulla, which contains the vasomotor area. Previously, the pontomedullary area has been mentioned as the part of the brain that is the most readily accessible to pyridinium oximes.[107-110]

Erdmann and Engelhard[123,124] reported that in vitro IV was a more potent reactivator of acetylcholinesterase inhibited by DFP or paraoxon than I or III. IV also reacted directly with these OP compounds, particularly paraoxon, at high rates. IV was found to enter the CSF more rapidly and to a greater extent than I and to be excreted in the urine comparatively slowly (11% during 6 h). The maximal concentration of IV in the blood of the rabbit occurred about 20 min after an intramuscular injection. Removal of the oxime from the blood between 1 and 4 h after an intravenous injection amounted to about 80% of the oxime present 1 h after the injection. After a lethal dose of IV, there was somnolence, paralysis of the extremities, and terminal paralysis of respiration. A single intraperitoneal dose of IV at 150 mg/kg resulted in diffuse fatty infiltration of the liver, which disappeared within 1-2 d. No other important pathologic effects were reported.

Dilute solutions of IV (36 mg/100 ml) were not irritating to the eyes.[124] An isotonic solution containing 10 times that concentration of IV was not hemolytic. A solution containing 3.6% of IV was not irritating to the hairless skin inside the ear of the rabbit. IV at 10^{-4} M increased the ability of acetylcholine to stimulate the rectus abdominis muscle of the frog; a concentration of 3×10^{-3} M paralyzed neuromuscular transmission in the isolated rat phrenic nerve-diaphragm preparation. The isolated jejunum of the rabbit was stimulated fleetingly by IV, but this oxime relaxed segments of jejunum that had been caused to contract by acetylcholine or carbamoylcholine. Intravenous injection of IV into a cat at 15 mg/kg decreased the response of the heart to stimulation of the vagus nerve and completely blocked contractions of the nictitating membrane in response to stimulation of the cervical sympathetic chain. An intravenous dose of 50 mg/kg decreased blood pressure and respiratory activity. When mechanical ventilation of the lungs was provided, cats survived doses of 200 mg/kg. Repetitive administration on 3 successive days of 50-70% of the LD_{50} resulted in muscular weakness, swayback, and exophthalmos. Daily injection during 30 d of 30% of the LD_{50} had no evident deleterious effect on the health of the animals.

IV has been found[125] to antagonize stimulation of the isolated ileum by carbamoylcholine and ethyl arecaidinate (a homologue of arecoline) and to cause parallel shifts of the dose-response curves of each of these agonists. This oxime was considered to be a functional antagonist of cholinergic agonists, rather than a competitive one, because various mixtures of IV with the competitive anticholinergic drug, N-methylatropinium nitrate, shifted the dose-response curves of the agonists by amounts that were not proportional to the sum of the individual displacements by the two antagonists. The parasympatholytic action of IV is suggested, therefore, to arise from contact between IV and a specific receptor for that molecule different from the molecule(s) with which the agonists and the N-methylatropinium salt interact, but functionally interdependent with it. This interdependence may involve an allosteric mechanism, but that has not been demonstrated.

METABOLISM OF OXIMES

2-PAM I in dilute hydrochloric acid (0.1-0.4 N) is hydrolyzed in a first-order reaction.[126] The rate of acid hydrolysis at a constant H^+ ion concentration had a C_{10} of about 2 when the temperature was increased from 37.8°C to 47.6°C. The hydrolysis of 2-PAM I was not catalyzed by hydroxyl ions, but was rapid at high pH. The *syn* form of the oxime was converted to the *anti* form more rapidly at a pH of 13 than at one of 7 or 1.

Within the range of pH from 1 to 13, 2-PAM I was found to have its maximal stability with respect to hydrolysis in aqueous solvents at a pH of 1, whereas III was most stable at a pH of 3.[127] Even at the indicated pHs, 30% of 2-PAM I and 20% of III were hydrolyzed before equilibrium was reached. After the oximes apparently reached an equilibrium with their hydrolytic products, degradation of the oximes continued at a low rate. Ellin[128] suggested the following sequence of reactions during degradation of 2-PAM I in aqueous solution: the oxime is dehydrated to *N*-methylpyridinium-2-nitrile (A), which is converted to *N*-methylpyridinium-2-hydroxylate (B) and then to *N*-methylpyridinium-2-one, hydrogen cyanide being released during the conversion of A to B.

Creasey and Green[129] reported that II is soluble in water to the extent of 1 g in 2 ml; like I, it is more stable in aqueous solution at low pH, the pH of maximal stability being 4-5. When II in a solution at a pH of 4.5 had been freeze-dried and stored in a sealed glass container for 6 mo, no cyanide was detectable in the container when it was opened. Similar treatment of a solution at a pH of 6.0 resulted in detection of cyanide by odor when the container was opened. Heat sterilization of an aqueous solution of II was found to result in the formation of small amounts of cyanide and in a change in the color of the solution from pale yellow to orangebrown. A homogenate of liver from the rat was found to decompose II slowly; other biologic samples (whole blood, kidney, skeletal muscle, urine, and feces) did not.

Kondritzer *et al.*,[130] in searching for a salt of 2-PAM that would be more soluble than 2-PAM I in water, made a number of other salts. Most of these were considerably more soluble in water than 2-PAM I. One of the most soluble was the lactate, with a solubility of 1 g/ml. Unfortunately, this salt was found to be quite unstable in aqueous solution with respect to heat. I was less soluble in water than the lactate, but more than 13 times as soluble as the iodide. Furthermore, solutions of I at a pH of 3.5-4.3 could be autoclaved at 120°C for 15 min with only a 4% loss. Solutions of this salt stored at 50-70°C for 1-3 mo contained at least 78% of the original oxime and no more than 0.1% of hydrogen cyanide. When the aged solutions were examined for lethality to experimental animals, their lethal activities were exactly those expected on the basis of their oxime concentrations.

Barkman[131] compared the stabilities of I and II at various pHs and storage temperatures. Storage of a solution of I at a pH of 3 and 50°C for a year resulted in some increase in pH and in loss of 37-60% of the oxime in the original solution. Similar storage of a solution of II at the same pH and at 45°C for 2 yr resulted in loss of 80% of the oxime. Storage of solutions of these two salts at the same original pH, but at a temperature of only 25°C, resulted in loss of only 5% of I during 1 yr and of 4.8% of II during 2 yr. The pH increased by 1.16 during storage of the solution of II at 45°C for 2 yr, but by 0.19 during storage of that of I at 50°C for 1 yr. The principal products of degradation of these oximes were found to be N-methylpyridinium-2-aminocarbonyl and N-methylpyridinium-2-nitrile. These end products have low toxicities, so the deteriorated solutions of I and II were less toxic than freshly made ones (see also Barkman, et al.[132]) If the temperature of storage of solutions of either of these 2-PAM salts can be kept below 25°C, the solutions should have shelf-lives of several years.

When stored at 10-20 °C, solutions of III had their greatest stabilities at an initial pH of 6.[133] When the temperature of storage was 30°C or more, the initial pH that provided the greatest stability of the oxime in an aqueous solution was 5. With that initial pH, the estimated half-life of III in solutions stored at 40°C was 9.6 yr. The half-life of I in solutions at the pH of greatest stability, initially 4, for storage at 40°C was estimated to be 9 yr. In a solution containing also atropine and benactyzine at a pH of 2.8, 1.4% of the protective potency due to III was lost after 1 yr at 25°C.[134]

There seems to be little basis for choice among I, II, and III on the grounds of relative stability in solutions, so long as the storage temperature can be held below 40°C and the initial pH of the solution is adjusted to the value that yields the greatest stability for the particular oxime. For the salts of 2-PAM in solutions with pHs above those of maximal stability, the sequence of hydrolytic products may be N-methylpyridinium-2-aminocarbonyl, N-methylpyridinium-2-nitrile, N-methylpyridinium-2-hydroxide, and N-methylpyridinium-2-one, hydrogen cyanide being a side product. At pHs at or below those of maximal stability, the end products of hydrolysis of N-methylpyridinium-2-formyl oxime are N-methylpyridinium-2-aldehyde and hydroxylamine. Information on the storage stabilities of IV and V has not been found, other than a note that, when stored at 37°C at an unspecified pH, both 2-PAM I and V had half-lives greater than 7 mo.

After oral administration of I to dogs at nearly 100 mg/kg in an aqueous solution, the concentration of oxime in the plasma 1 h later was as great as was measured at any later time.[135] By 5 h after the dose, the plasma concentration had decreased to 51.3% of that at 1 h; by 13 h, it was only 18% of that at 1 h. When approximately the same dose was given in tablets, the peak plasma concentration did not occur until 2 h later; it was more than 2.3 times the plasma concentration found 1 h after administration of the solution. This last finding suggests that the oxime is absorbed rapidly from a solution introduced into the gastrointestinal tract, so that the peak plasma concentration of oxime after oral administration of the solution may have passed before the first blood sample was taken 1 h after administration of the solution. By 5 h after administration of the tablets, the plasma concentration was slightly more than 39% of the peak concentration, but still nearly twice that measured at the same time after administration of I in solution. By 13 h after the tablets had been given, the plasma concentration was only 7.6% of the peak concentration and was the same as that measured at the same time after the solution had been administered. Inclusion of atropine in the preparation of the oxime was found not to influence absorption of the oxime. Administration of a nearly lethal dose of sarin to rabbits before the intramuscular injection of II or III nearly doubled the mean time (9 min vs. 5 min) for attainment of the peak plasma concentration.[136] The concentration of III in the blood was nearly twice that of II when equal doses of the two oximes were administered. However, Erdmann and Okonek[137] found that introduction of parathion into the lumen of a dog's intestine increased the absorption of IV from the lumen. In the rat, oral administration of disodium EDTA with IV nearly doubled the rate of absorption of the oxime. The peak plasma concentration of oxime at 1 h after administration of that mixture ranged from 6.9 to 17.4 μg/ml.

Sundwall[138] regarded a concentration of oximate ion in the blood of 4 μg/ml as minimal to overcome the bradycardia and apnea induced by lethal doses of isopropoxymethylphosphorylthiocholine in an anesthetized cat. Kunkel et al.[139] found that three of six rabbits given intramuscular injections of atropine sulfate at 6 mg/kg 55 min before intravenous injection of soman at 1.5 times the LD_{50} lived for 7 d thereafter. When the atropine was accompanied by I at 15 mg/kg, yielding a plasma concentration of oxime of about 4.8 μg/ml, two of six rabbits lived for 7 d. When the dose of atropine sulfate was increased to 10 mg/kg and the time between prophylaxis and poisoning was shortened to 5 min, only one of six rabbits challenged with soman at 2 times the LD_{50} lived for 7 d. When I at 15 mg/kg accompanied the same dose of atropine, the plasma concentration of the oximate ion just before soman was administered was about 25.4 μg/ml, and three of six rabbits given intravenous injections of soman at 2 times the LD_{50} lived. Larger doses of I, up to 45 mg/kg, yielded a plasma concentration of oxime of about 50 μg/ml and increased survival after soman at 2 times the LD_{50} to four of six. Accepting that lethal intoxication with soman is unusually difficult to prevent or treat, one nonetheless sees in these results an indication that the plasma concentration of oxime is important in determining the severity of intoxication by OP compounds that can be withstood.

Jager et al.[59,115] reported that infusion of 2-PAM I into dogs at 0.5 mg/kg per minute resulted after about 2 1/2 h in a relatively stable serum concentration of oxime of about 40 μg/ml. Ligation of the renal pedicles resulted in a rapid increase, during the 30 min thereafter, in the serum concentration of oxime to nearly 120 μg/ml. In one dog in which the infusion was continued for another 90 min, the serum concentration rose to nearly 140 μg/ml. In nephrectomized rats given intravenous 2-PAM I at 50 mg/kg during 2 min, the serum concentration of oxime decreased during an hour at about two-thirds the rate at which it decreased in control rats. Furthermore, the extrapolated serum concentration of oxime in the nephrectomized rats at zero time was one-third greater than that in the control rats. Evidently, therefore, the kidneys are important organs for the removal of 2-PAM salts from the circulating blood.

These investigators found also[59,115] that homogenates of rat liver were able to degrade both 2-PAM I and V if oxygen was present during the incubation; anaerobic incubation left the concentrations of the oximes in mixtures with the homogenate the same as they had been before incubation. Dultz et al.[23] found that dog kidneys took up from the blood more than 5.4 times as much 2-PAM I as V and that V disappeared from the serum of nephrectomized rats at essentially the same rate as from that of normal rats. Excretion into the urine seems to be a much more important route for removal of 2-PAM salts than of V from the body.

On comparing various organs of the dog other than the kidneys for ability to take up bloodborne 2-PAM I and V, Dultz et al.[23] found that V was abstracted from the blood by the brain slightly more than 11 times as readily as 2-PAM I, whereas liver, spleen, skeletal muscle, and cardiac muscle removed the two oximes in ratios between 1.0 and 1.7. Fat took up 4.5 times as much 2-PAM I from the blood as V. Because of its comparatively great ability to enter the brain, V has been considered by some people to be an especially good antagonist of inhibition of brain cholinesterase by OP compounds.

Kaiser[41] found that excretion of the label in intravenously injected 2-PAM I having ^{14}C in the methyl group attached to the N atom in the pyridine ring was largely in the urine of mice; the feces accounted for less than 5% of the total excretion. The label was excreted rapidly (38.7% in 145 min) in the urine of one cat. The urine of mice was believed to contain at least six chromatographically separable metabolites of 2-PAM I, in addition to the unchanged oxime, whereas the urine of the one cat seemed to contain only unchanged oxime. Only 0.18% of the label was found in the expired air of mice during 6 h after intravenous injection of labeled oxime. The label disappeared from the blood of the cat rapidly: by 2.5 h after injection of the oxime, only about 17.5% of the peak concentration of the oxime in the plasma remained there. Correspondingly, about 38.8% of the label had been excreted in the urine during the same time. In the cat, comparatively large concentrations of the label were found in the gall bladder and bile, the urinary bladder, the small intestine, the feces, and the liver.

Crook et al.[57] reported that the plasma concentration of III after its injection into a dog at 10 mg/kg followed essentially the same course as that of 2-PAM I injected intravenously at 30 mg/kg. Inasmuch as the plasma concentrations at approximately the same times after injection of the two oximes were almost identical, despite the difference between the doses, one must assume that III has a much smaller volume of distribution than 2-PAM I and that either the kinetics of removal of 2-PAM I from its larger volume of distribution were correspondingly greater than those of III or the 2-PAM I in extravascular components of its volume of distribution was held there tenaciously. The fairly rapid changes in the 2-PAM I concentrations found by Kalser[41] in various organs and tissues of the mouse argue against the validity of the latter possibility.

Enander, Sundwall, and Sörbo[140-142] found that either oral or intramuscular administration of II to rats at 500 or 100 mg/kg, respectively, increased by many times the urinary excretion of thiocyanate. From the amount of thiocynanate excreted above the base line, the quantity of hydrogen cyanide produced in metabolism of the oxime was calculated to be 0.1 mg/kg--a little more than one-third the LD_{50} for rats by intraperitoneal injection. Urine from rats given 120 μmol of II intramuscularly or 400 μmol by mouth contained 3.9-7.8% N-methylpyridinium-2-nitrile methanesulfonate. When this compound was injected intramuscularly into rats at 90 mg/kg, thiocyanate was excreted in the urine in increased amounts. Also present in the urine was a metabolic product that yielded cyanide on acidification of the urine, similar to a cyanide-yielding metabolite of II found earlier.

Studies with N-[^{14}C]methylpyridinium-2-aldoxime iodide administered to rats by intramuscular injection at 40 or 100 mg/kg or by mouth at 100 mg/kg revealed that about 87% of the label was excreted in the urine during the 24 h after injection, but that only 52% was excreted during the same time after oral administration. The label excreted in the urine during this period was largely (80-90%) in the form of unaltered oxime. Other metabolic products identified in the urine were N-methylpyridinium-2-nitrile and N-methylpridinium-2-carboxylic acid. Two other peaks in the radio-chromatograms of the urine were thought to represent N-methyl-α-picolinium amine and the cyanide-yielding metabolite mentioned earlier, but the identities of the compounds were not established, because there was too little of the substances represented by these radiochromatographic peaks. No N-methylpyridinium-2-one was found. Enander et al.[142] presented a scheme for the metabolic transformations undergone by 2-PAM whereby the oxime is converted to either the nitrile, the aminocarbonyl derivative, or the aldehyde. The aminocarbonyl compound and the aldehyde were considered to be converted to the carboxylic acid as the end product. End products thought to be derived from the nitrile are the pyridone and the unidentified cyanide-yielding metabolite.

Way criticized this work as being superficial and inept and developed a chromatographic method for removing from a reaction mixture or solution all compounds containing a pyridinium group. The pyridinium-containing compounds could then be resolved at pHs permitting the molecules to exist without degradation. Using this method, Way et al.[143-146] identified N-methylpyridinium-2-nitrile, N-methylpyridinium-2-one, and N-methylpyridinium-2-aminocarbonyl-4-one as metabolic products of 2-PAM in the perfusate from isolated rat livers. They also found, but did not identify, an N-methylpyridinium-2-O-conjugate, which was not hydrolyzed by either β-glucuronidase or phenol sulfatase. When exposed to 0.3 N NaOH, the conjugate released N-methylpyridinium-2-one. N-methylpyridinium-2-ethoxy iodide was synthesized and found to yield the pyridone on exposure to 0.3 N NaOH. However, the metabolic product and the synthetic compound were found to have different mobilities on paper chromatograms with various solvent systems. The synthetic compound may be a model of the natural conjugate, but is not identical with it.

Way presented a scheme for the metabolism of 2-PAM wherein the oxime is converted to either a 2-nitrile-4-one or a 2-acetiminomethyl or a 2-phosphoryliminomethyl compound. The acetimino or phosphorylimino compound is converted to the 2-nitrile, which is changed into either the 2-cyanohydrin or the 2-nitrile-4-one derivative. The 2-nitrile-4-one is metabolized to the 2-aminocarbonyl-4-one. The 2-cyanohydrin is proposed as the precursor of the 2-one derivative and also of the 2-O-conjugate through a 2-cyano-O-conjugate. Both reactions of the cyanohydrin would release CN^- ion.

Way et al.[145] found N-methylpyridinium-2-nitrile in human urine as a metabolite of 2-PAM. Kramer[147] reported that five men given 2-PAM I at 2 g/day by mouth on 2 consecutive days while they were on a closely supervised regimen of dietary and other intakes excreted in their urine not only unaltered 2-PAM, but also a material that had a bright blue fluorescence when exposed to UV light (366 nm). This material was shown not to result from simple exposure to urine by incubation of 2-PAM I in normal human urine under several different conditions for up to 7 d. It was thought possibly to be a derivative of N-methylpicolinic acid.

Berglund et al.[148] studied the handling of II by the dog's kidneys and found that the amount of the oxime excreted always was greater than the amount filtered through the glomeruli. At a plasma concentration of II in one dog of about 6.2 μg/ml, the difference between the amount excreted in the urine and the amount filtered was about 1.2 mg/min in a total excretion of about 1.75 mg/min. This plasma concentration of the oxime gave the Tm for tubular secretion in that animal (the Tm was reached in other dogs at plasma concentrations of II of 6-8 μg/ml). Loading dogs with ammonium chloride (6 μg/d by mouth for 3 d) before the experiment increased excretion of the oxime without altering appreciably its filtration. Conversely, induction of alkalosis by intravenous infusion of 0.6 M sodium bicarbonate decreased excretion of the oxime without changing significantly its filtration. Alkalinization of the urine by administration of acetazolamide had no effect on the urinary excretion of the oxime. Probenecid (priming with 25 mg/kg followed by infusion at 40 mg/kg per hour) lowered the urinary excretion of the oxime from 1.66 mg/min to 1.61 mg/min and tubular secretion from 0.96 mg/min to 0.88 mg/min.

These differences are not statistically significant. Apart from a conclusion that the tubular epithelium has a more effective transfer system for the oximate form of II than for the N-oxide form, the meaning of these findings is obscure.

The only other oxime on which any metabolic information was found is III. Way et al.[149] perfused III labeled with ^{14}C in the 1 and 3 positions of the 3-carbon chain between the pyridinium moieties through isolated rat livers. The only metabolite that they reported finding in the perfusate was 1-(4-aldoximinopyridinium)-3-(4-cyano-pyridinium) propane ion, which was identified by comparison with the authentic synthetic compound. Later, DeMiranda et al.[150] found the same compound as the principal metabolite in the urine of rats that had been given doses of III. III seems to be treated metabolically as though it were one molecule of 2-PAM attached to a large inert group.

In an attempt to find a way of extending the protective action of oximes, Stern and Bosković[151] explored the possibility that an inhibitor of drug processing enzymes, diethylaminoethyl diphenylpropylacetate (SKF 525A), can increase the ability of oximes to antagonize the lethal actions of tabun. In the scheme used, SKF 525A and atropine were injected into experimental animals 40 min before tabun was injected; oximes were injected 10 min before tabun. SKF 525A approximately doubled the protective activity of mixtures of atropine with 2-PAM I, III, and a mixture of 2-PAM I and V. Later, Milosević and Terzić[152] compared other inhibitors of microsomal enzymes with SKF 525A for potency in increasing the ability of III to prevent the lethality of paraoxon. The principal difference between III alone and with SKF 525A was that SKF 525A significantly prolonged the time between injection of III and of paraoxon at which the oxime would remain protective. For example, injection of III at 25 mg/kg 30 min before paraoxon yielded 30% survivors in the group. When the same dose of III was injected with SKF 525A at 50 mg/kg 60 min before the same dose of paraoxon, all the animals lived. These investigators found also that one of a group of related inhibitors of microsomal enzymes, diethylaminoethyl phenyldiallylacetate (CFT 1201), was at least as effective as SKF 525A in a much smaller dose.

The same people carried the study of CFT 1201 further.[153] When rats were given CFT 1201 1 h before intravenous injection of III, the blood concentration of III was only 21.6% greater than when III was administered alone. When mice were given oximes subcutaneously and CFT 1201 intraperitoneally simultaneously 15 min before a challenge with paraoxon at 3 times the LD_{50}, the protective dose of 2-PAM I was 24.3% of that when 2-PAM I was given alone, and that of III was 20% of that when III was injected alone. CFT 1201 and SKF 525A alone had almost no protective actions against tabun or paraoxon.

STUDIES WITH HUMAN SUBJECTS

Jager et al.[59,115] injected 2-PAM I intravenously into five human subjects during 2-4 min at 15 mg/kg and I into five other subjects at the same dose. One patient with a plasma concentration of urea nitrogen of 165 mg/100 ml was given the same dose of 2-PAM I. During 4 h after these doses, essentially all the 2-PAM I left the serum of the five normal subjects, whereas the serum concentration of this oxime in the azotemic patient decreased by only about 8.6%. The mean concentration of V in the serum of normal subjects decreased by about 13.5% during 4 h. Correspondingly, three subjects given 2-PAM I excreted in their urine a mean of 80.9% of their doses of the oxime during the 6 h that followed the injections, whereas two subjects given V excreted a mean of only about 6.8% of their doses of that oxime during the 6 h after administration of the oxime. Urinary excretion of 2-PAM I by the azotemic subject seems not to have been measured.

During a continuous intravenous infusion of V into a subject at 0.27 mg/kg per minute, samples of spinal fluid taken at 60 and 150 min after the start of the infusion contained a mean of 68.3% ± 2.4% of the serum concentration of the oxime at the same time.[115] A subject given an infusion of 2-PAM I for 60 min at 0.73 mg/kg per minute had a serum concentration of the oxime of 2.8 mg/100 ml at the end of the infusion, but had no detectable concentration of that oxime in the spinal fluid. These findings corroborate the idea that V may have readier access than the pyridinium oximes to the brain.

In 1964, Calesnick[154] and Calesnick et al.[155] reported that, when I was injected intravenously into normal male and female volunteers at 15 mg/kg (three subjects), both systolic and diastolic blood pressures were increased immediately after the end of the infusion (usual duration, 15 min). The hypertension lasted for 1.5-4 h and was accompanied by a slight increase in heart rate. The plasma concentration of oxime at the end of the infusion ranged from 12.3 to 17.3 µg/ml. Two subjects given similar infusions of I at 30 mg/kg had no immediate increases in blood pressure, but the systolic and diastolic pressures rose slowly--e.g., to about 32 and 44 mm Hg above the original pressures, respectively, at about 35 min after the end of the infusion in one subject; subnormal blood pressures developed about 3 h later. The subjects given the smaller dose of I also developed subnormal pressure after the hypertensive phase of the response. The mean plasma concentration of I at the end of the infusions at 30 mg/kg was 17.6 ± 0.3 µg/ml. Infusions of I at 45 mg/kg into two subjects resulted in marked increases in systolic and diastolic blood pressures immediately after completion of the infusions. The hypertensive response lasted for about 1.5 h and was followed by the development of subnormal blood pressure about 2.5 h later. Increases in the voltage of the T waves and in the length of the PR interval of the ECG were reported after this dose. Administration of a second dose of I after the blood pressures had returned to the control values yielded a second hypertensive response. Ephedrine intensified the hypertensive response to I, whereas phentolamine and reserpine reduced it.

Injection of I intramuscularly at 15 mg/kg into two subjects gave a plasma concentration of oxime of 4 µg/ml, but had no effect on blood pressure.[154] Two other subjects given intramuscular doses of 30 mg/kg developed hypertension about 1.5-2 h after the end of the infusions. A subject who inhaled aerosols of 10% and 50% aqueous solutions of I did not develop hypertension or detectable oximemia. Oral administration of 1 or 2 g every 6 h for 5 d to eight subjects resulted in no significant changes in blood pressure or heart rate.

Infusion of II at 45 mg/kg into one subject resulted in a hypertensive response immediately after the infusion was completed.[154] Oral doses of 2 and 4 g of II had no detectable effect on blood pressure in another subject.

When 4 g of III were given by mouth to one subject, there were slight decreases in systolic and diastolic blood pressure 6 h later. Infusions of III at 15 or 30 mg/kg into one subject produced immediate, brief hypertensive episodes at the end of the infusions, followed by a period of subnormal pressures lasting from about 22 min to more than 363 min after the end of the infusion for the systolic pressure; the diastolic pressure did not fall below normal until about 117 min after the end of the infusion, but was still below normal at 363 min after completion of the infusion.

More detailed data from these experiments and from others in which I and 2-PAM maleate were administered orally and by intraduodenal tubes, to compare rates of absorption of the two salts by the two methods of administration, were given by DiPalma and Calesnick.[156] As a result of these studies, Calesnick _et al._[157] concluded that I was more satisfactory than II or III, in that the last two oximes caused marked gastrointestinal disturbances that limited both the amounts of these oximes that could be administered and the durations of their administration that could be tolerated. This limitation was especially marked with III, which also caused nervousness, malaise, dizziness, paresthesia of the face and arms, rash, and icterus in some subjects.

Details of studies in which propranolol and phentolamine were found to antagonize the hypertensive effect of infusions of I at 25-45 mg/kg were given in the same report by DiPalma and Calesnick.[157] Doses of 5 mg of phentolamine, probably as the methane sulfonate although they did not identify the salt used, and of 4 or 5 mg of propranolol were used in four subjects (one woman and three men) aged 23-32 yr. In three subjects given I by intravenous infusion at 25 and 30 mg/kg during 15 min, phentolamine was the more potent antagonist of the hypertensive effect of I; in one subject given I at 45 mg/kg, propranolol was a better antagonist than phentolamine. It is of some interest that the adrenergic blocking agent, phentolamine, usually had a greater antagonistic action against the hypertensive effect of I than the nonselective β-adrenergic blocking agent, propranolol. This suggests that the principal action of I is directly on the vascular smooth muscles--a posssibility that is supported by the finding that the hypertensive action of I usually does not involve a marked change in heart rate.

Kondritzer et al.[158] administered I, 2-PAM lactate, 2-PAM dihydrogen phosphate, II, 2-PAM I, and III to human subjects as aqueous solutions. The subjects were primed with 400 ml of water during the hour before they drank the solution of oxime and drank 100 ml of water during each of 5 consecutive hours after taking the oxime. The peak plasma concentrations of oxime occurred at about 2 h after drinking of a solution of 2-PAM I that contained a mean dose of 71.4 mg/kg, at about 2.5 h when the dose was increased to 114.3 mg/kg, and at about 3 h when the dose was increased further to 142.9 mg/kg.

The lactate gave a greater peak concentration of oxime in the plasma than the same dose of the dihydrogen phosphate, but required a longer time to induce it (3 h vs. 2 h); the peak concentrations differed by 18.9% of that established by the dihydrogen phosphate, although the molecular weight of that salt was greater than that of the lactate by only 3.4% of its own molecular weight. When I and II were administered in equimolar doses, the peak plasma concentrations of oxime were reached ater 2 h. With doses of 0.31 mmol/kg, II gave a peak concentration greater than that given by I by about 4.6 M. When the dose was 0.12 mmol/kg, the peak concentration established by I was about 1.5 μM above that established by II. These two salts are probably absorbed and eliminated from the plasma at closely similar rates.

The biologic half-lives of the five salts of 2-PAM calculated from both the data on plasma concentrations at various times after ingestion and those on urinary excretion are given in Table 4. The table demonstrates that the iodide was cleared from the plasma more slowly than the other salts and that the dihydrogen phosphate was cleared from the plasma a little more rapidly than the others.

The only undesirable effects observed during these studies were signs and complaints of iodinism by the subjects given 2-PAM I and decreases in red-cell and plasma cholinesterase activities of about 20% in the subjects given III. Less of III was excreted in the urine (3% during 24 h) than of the monoquaternary compounds (27% in 14 h).

Sidell et al.[76] gave oral doses of 3-9 g of I to a total of 28 men. Although these doses were not adjusted to the body weights of the subjects, there was a general tendency for the mean peak plasma concentration of oxime to increase as the dose increased. The mean peak concentration varied from 4.20 μg/ml after 3 g of I was ingested by four subjects to 9.15 μg/ml after 9 g was taken by two subjects. The time for attainment of the peak concentration varied from 30 min to 3 h, without any discernible relation with dose or any other variable in the experiment. The mean half-life of the oxime, calculated from concentrations measured in the plasma at various times, was 2.66 h in 25 subjects; the mean half-life calculated from the amounts of oxime excreted in urine at various times was 2.44 h in 21 subjects. In five experiments in which the mean peak plasma concentration of oxime of 6.1 μg/ml was attained 2 h after ingestion, the mean 6 h after ingestion was 2.0 μg/ml--slightly less than 32.8% of the mean peak concentration.

When these investigators[76] administered oral doses of I every 6 h for 48 h, the plasma concentration of oxime proceeded as a series of peaks at 3, 9, 15, 21, 27, 33, 39, 45, and 51 h. There was a tendency for each peak to be slightly greater than the preceding one. When the interval between doses was shortened to 4 h, the peak concentrations were about 1.3 times those when the same dose was given every 6 h.

TABLE 4

Biologic Half-Lives of 2-PAM Salts Given Orally to Man

	Half-Life, h	
2-PAM Salt	Concentration in Plasma	Based on Urinary Excretion
Iodide	2.0 (9)	2.2 (5)
Chloride (I)	1.7 (7)	1.7 (7)
Methane sulfonate (II)	1.7 (10)	1.6 (10)
Lactate	1.7 (9)	1.7 (9)
Phosphate	1.3 (5)	1.5 (5)

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[a] Figures in parentheses are numbers of subjects.

Sidell _et al._[159] found that, when doses of 600 mg of I and 2 mg of atropine sulfate were injected intramuscularly simultaneously but separately into the two arms of a man, his heart rate rose more sharply and to a higher value than when the two compounds were injected in one solution. The mixed solution seemed to produce less pain than intramuscular injection of I alone. On the basis of this study, there is no evident disadvantage in mixing atropine and I in a single solution for intramuscular injection. There is a question, however, whether the doses of atropine and I should be linked in a fixed ratio, as they would be if these two compounds were provided only as a mixed solution.

Swartz and Sidell[160] administered I to subjects under various conditions of ambient temperature and exercise (walking on a treadmill). Resting at 21°C was the baseline condition. The renal clearances of creatinine, _p_-aminohippurate, and I were measured during rest at 40.6°C, during walking at 3 mph during 20 of each 30 min in a period of 3 h at 21°C, and during walking at 3 mph during 20 of each 30 min in a period of 3 h at 40.6°C. Whereas the renal clearance of creatinine was decreased progressively by exercise, by exposure to an increased ambient temperature, and by exercise at the higher ambient temperature, the clearances of both _p_-aminohippurate and I were increased during rest at the higher ambient temperature. Walking on the treadmill in the heated room, however, decreased the clearances of _p_-aminohippurate and I even more than walking on the treadmill at the lower temperature. That the effects on these two renal clearances were similar is not astonishing when one considers that tubular secretion is the most important part of the mechanism of clearance of both substances from the plasma. It is likely that exposure to the higher temperature at rest resulted in increased blood flow to the kidneys by vasodilatation, that exercise at the lower ambient temperature decreased blood flow to the kidneys by shunting of blood to the active muscles and possibly to the skin to permit dissipation of the extra heat produced during the exercise, that the greater heat load imposed by the combination of higher ambient temperature and exercise resulted in an even larger shunting of blood away from the kidneys to the skin, and that the resultant of vasodilatation in the active skeletal muscles and in the skin was a sharply decreased blood flow to the kidneys and, consequently, lower tubular secretion of _p_-aminohippurate and I.

After being found to be healthy on a thorough physical examination accompanied by a broad range of laboratory examinations, 22 men were used in studies of the renal clearance of I, after intravenous injection at 5 mg/kg under a variety of conditions.[161] Alkalinization of the urine to a pH above 7.5 by administration of bicarbonate and acidification of the urine to a pH below 5.0 by administration of ammonium chloride both reduced urinary excretion of I. When 200 mg of thiamine was injected intramuscularly 20-30 min before intravenous injection of I, urinary excretion of I during the 5 h after its injection was decreased to the greatest extent (by almost 24%). Thiamine resulted in an increase in the total volume of distribution of I of about 47.4%, the expansion of the central volume being about 13.3% greater than that of the peripheral volume. The mechanism of the changes in the volume of distribution of I is not apparent.

During the first 3 h after intravenous injection of I that followed administration of thiamine, urinary excretion of the oxime was about 12.7% below that during the corresponding period of the control experiment; during the remainder of the run, it was 62.2% above that during the same period of the control experiment. Inasmuch as intravenous injection of 900 mg of sodium p-aminohippurate with I decreased by only 6.3% the urinary excretion of I during the first 3 h after its administration, the tubular transport mechanisms for I and for p-aminohippurate probably are different.

Josselson and Sidell[162-164] extended study of the effect of thiamine on the pharmacodynamics of I in the human body. They found initially[162] that an infusion of thiamine at 100 mg/h during 2.5 h led to a greater plasma concentration of oxime after intravenous injection of I at 5 mg/kg than when the injection of I was not accompanied by an infusion of thiamine. The renal clearance of I and the urinary excretion of I were greater during the first 1.5 h of infusion of thiamine than during the corresponding period after injection of I without infusion of thiamine. The investigators concluded that thiamine decreased the peripheral volume of distribution of I in this study. However, their published value for the standard deviation of the mean for this variable during the infusion of thiamine was 0.01 times the real value; that may have contributed to this unjustified conclusion. The sole barely significant change in the volume of distribution of I was an increase in the central volume of distribution. The most significant changes in the men given thiamine infusions were a decrease in the removal of I from the central compartment of the volume of distribution, a decrease in the movement of I from the central to the peripheral compartment, and a decrease in the value of the exponential constant for the rapid phase of the decrease in the plasma concentration of I.

Infusions of thiamine at two different rates during intravenous injections of a constant dose of I produced sequentially slower removal of I from plasma.[163] The half-time for the rapid phase of removal of I from plasma was 4.2 min in the control runs; it was increased to 8.4 min during the infusion of thiamine at both rates. The half-time for the slow phase of removal of I from plasma was 59.8 min in the control runs; it was increased to 87.6 min and 91.2 min, respectively, by the lower and higher rates of infusion of thiamine. The rate constant for removal of I from the central compartment was 2.94 in the control runs and was decreased to 1.32 by infusion of thiamine at the lower rate; the higher rate of infusion of thiamine (twice the lower one) induced no significant further reduction of this rate constant. The rate constant for movement of I from the peripheral compartment to the central one was 4.95 in the control runs; it was decreaed to 2.49 by the lower rate of infusion of thiamine, but rose to 3.21 with the higher rate of infusion. The last value had a very large standard deviation and consequently was not significantly different from either the rate during the control runs or that during infusion of thiamine at the lower rate. The higher rate of infusion of thiamine, but not the lower one, decreased significantly the peripheral and total volumes of distribution of I. The higher rate of infusion of thiamine markedly prolonged side effects of the oxime, including lethargy, but was tolerated well by the subjects.

Infusions of 1 g of I, with and without 200 mg of thiamine hydrochloride, into five men during 30 min produced hypertension in two subjects during the infusions that included thiamine.[164] In the trials without thiamine, the only effects detected by the investigators or reported by the subjects were transient blurring of vision, diplopia, and a sensation of expansion of the eyeballs, never lasting for more than about 3-5 min, in four of the five subjects. During the infusions that included thiamine, in addition to the hypertension in two subjects, one other subject had an increased heart rate, and all complained of the same visual effects that were reported during the control infusion. These were more marked and lasted for up to 2 h, instead of for a few minutes as during the control runs. Four of the men also complained of fatigue and drowsiness; two complained of "vitamin gustatory sensation."

The two men who had hypertension after the infusion of I with thiamine had mean increases in systolic blood pressure of 34 mm Hg and in diastolic pressure of 24 mm Hg. The mean maximal increase in heart rate of these two men and of the one other man who had only an increase in heart rate was 25 beats/min. In all five men, the mean plasma concentration of oxime was always greater in the experiments in which the infusion included thiamine than in those in which the infusion contained I alone, by about 1-11 μg/ml. During the first 1.5 h after the beginning of the infusions, those with I alone excreted in their urine a mean of 73% of the total 24-h excretion of the oxime, whereas when the infusions included thiamine only 34% of the 24-h excretion of the oxime took place during the first 1.5 h after the beginning of the infusions. During the first 3 h after the beginning of the infusions, 89% of the total 24-h urinary excretion of I given alone occurred, compared with only 55% of that of I accompanied by thiamine. During the remainder of the 24 h after the start of the infusions, 11% of the cumulative urinary excretion of I not accompanied by thiamine appeared in the urine, compared with 45% of that of I mixed with thiamine. The total urinary excretion of I during the 24-h collection period was 83.6% of that infused alone and 72.3% of that given with thiamine. It is unfortunate that these investigators never measured the excretion of thiamine alone and accompanied by I, because such information would allow one to judge whether I and thiamine use the same transport system across the tubular epithelium. This is a distinct possibility suggested, but not proved, by these studies.

Walker[135] reported the results of giving 12 subjects I orally in tablets at a mean of 58.6 mg/kg (experiment performed by G. Marier, P. Dussault, and J.M. Orr). Blood samples taken at intervals thereafter contained the following mean concentrations of I: 1 h, 6.48 μg/ml; 1.5 h, 6.33 μg/ml; 2 h, 6.75 μg/ml; 3 h, 5.77 μg/ml; 5 h, 3.03 μg/ml; and 7 h, 1.60 μg/ml. The mean time to the maximal plasma concentration of I was 1.67 h, and the mean value of that concentration was 7.28 μg/ml. The mean half-life of I in the plasma after reaching the maximal concentration was 125 min.

Sundwall[138] found that plasma concentrations of II established by intravenous and intramuscular injections of that oxime became approximately identical about 1 h after the injections, although the courses during that hour were quite different--one descended, at first sharply and then progressively more slowly, and the other ascended to a maximum and thereafter descended slowly. An intramuscular injection of a 25% solution of II caused some pain at the site of injection; similar administration of a 12.5% solution did not elicit pain. When 10 subjects got intramuscular injections of II at 30 mg/kg, three gave evidence of rapid absorption of the oxime, its plasma concentration rising to about 20 μg/ml within 5 min. The other ⑦ men seemed to have slower absorption of the oxime, its plasma concentration rising to 12-27 μg/ml after 20-30 min. In one subject, the peak concentration in the plasma was not achieved until 90 min after the injection. On oral administration of II at 45 mg/kg in gelatin capsules, the peak plasma concentration of about 5 μg/ml was reached after a mean (six subjects) of 130 min.

Sidell *et al.*[75] used two types of tablets containing II--one designed to disintegrate rapidly in the gastrointestinal tract and the other intended to provide slow, sustained release of oxime. The time courses of the plasma concentrations of the oxime with the two types of tablets were fairly similar, the peak concentrations being attained at the same time after ingestion. The peak concentrations after the rapid-release tablets were somewhat above those produced by the same total amounts of II in the sustained-release tablets and the descent from the peak concentration was less precipitous after the sustained-release tablets than after the rapid-release ones. Oral doses of I (form not specified) produced peak plasma concentrations of oxime sooner after ingestion than after ingestion of the tablets of II; the peak plasma concentration from a given dose of I usually was larger than that from a similar dose of II in the form of either of the tablets. The mean half-time for elimination of II from the body, starting at the peak plasma concentration, was 144 min for the rapid-release tablets and 135 min for the sustained-release tablets. The mean biologic half-life, calculated from analyses of urine, were 132 min for the rapid-release tablets and 138 min for the slow-release ones.

Holland et al.[165] performed a similar study, using a single dose of 4 g of II, whereas Sidell et al.[75] had used doses of 3, 5, 7, and 9 g. The two types of tablets used in these two studies were made by Glaxo Laboratories Ltd. Holland et al. used a total of 51 subjects and found that the rapid-release tablets yielded more rapidly (by about 1 h) a slightly larger peak plasma concentration of oxime (means for 42 subjects: 6.63 and 6.32 μg/ml for the rapid-release and slow-release tablets, respectively) and that the decrease from the peak concentration after the slow-release tablets was slower than that after the rapid-release ones. They concluded that use of a mixture of the two types of tablets would be preferable to use of only one type in facilitating both a rapid increase in plasma concentration of oxime to an effective point and a relatively prolonged maintenance of such a concentration. In a later paper, Holland and Parkes[166] reported that an intramuscular injection of 500 mg of II is needed to establish an effective plasma concentration. They found that three such doses given 20 min apart could be tolerated by normal people without complaints of visual disturbances. When intramuscular injection of II was added to an oral prophylactic regimen with II (4 g every 6 h), complaints of a feeling of enlargement of the eyeballs, blurred vision, and difficulty in accommodation after sudden head movement were expressed. Such complaints were more common from the subjects who received the larger doses of II.

Sidell et al.[167] used 20 subjects in a study of tablets of II prepared by Philips-Duphar in the Netherlands. Fourteen subjects took the tablets on empty stomachs; the other six ate a breakfast of eggs, bacon, and toast about 30-45 min before they took the tablets, which were coated with a methacrylate ester. Doses of 2, 4, 6, and 8 g of II were taken by the fasted subjects, and doses of 4 and 6 g by those who had broken their fasts. Peak plasma concentrations of the oxime were reached within 2-3 h after the tablets were ingested. The ingested doses resulted in the following order of decreasing peak plasma concentrations of oxime: fed, 6 g; fasted, 8 g; fasted, 6 g; fed, 4 g; fasted, 4 g; and fasted, 2 g. The only surprise in this list is the high positions held by the two groups of fed subjects.

Urinary excretion of II by the fed subjects was greater than that by the fasted subjects. The plasma concentration of II was a linear function of the logarithm of the dose of oxime for both groups of subjects, but the slope of the line for the fed subjects was greater than that for the fasted subjects. Although the difference was never large, the mean volume of distribution of II in the fasted men was greater than that in the fed ones. The mean first-order rate constant for absorption of II by the fasted subjects was greater than that for the fed subjects, and the mean first-order rate constant for elimination of II was greater for the fasted subjects than for the fed ones. Comparison of the plasma concentrations of II at various times after ingestion of the Netherlands tablets with those after ingestion of the English tablets reveals that comparable doses of II in the English tablets produced the lower plasma concentrations according to the data provided by Sidell *et al.*,[75] but the higher concentrations according to the data of Holland *et al.*[165] Values measured after the rapid-release English tablets were used for these comparisons. Because more closely similar methods were probably used in the two studies by Sidell *et al.*, comparison of the two sets of tablets based on the data of the two reports by Sidell *et al.*[75,167] may be more valid than that based on the data of Holland *et al.*[165] and of Sidell *et al.*[167]

Simon *et al.*[81] presented data that indicate, by a small extrapolation, that an oral dose of 4 g of IV in tablets would induce a peak plasma concentration of oxime in a human subject of about 6.2 μg/ml. This is about the same figure found by Sidell *et al.*[167] after 4 g of II in the Netherlands tablets, somewhat less than that reported by Holland *et al.*[165] after 4 g of II in the English tablets, and almost 1.5 times that found by Sidell *et al.*[75] with the English tablets.

Sidell and Groff[77] gave 10 men intramuscular injections of IV at 2.5, 5.0, 7.5, or 10 mg/kg. These doses produced mean peak plasma concentrations of the oxime of 12.1, 19.8, 34.9, and 39.3 μg/ml. These concentrations were achieved after 20-30 min. The logarithm of the peak concentration was a linear function of the logarithm of the dose injected. The half-time of removal from the plasma was about 81.3 ± 4.3 min. The half-time of urinary excretion was about 84.5 ± 10 min. Dose-related increases in the heart rate and systolic and diastolic blood pressures were also produced by IV. A comparison of the dose-plasma concentration relationships for IV and I reveals that the plasma concentration achieved by a given dose of oxime is 4.7-8.0 times as great for IV as for I. An apparently all-or-nothing symptom complex induced in some subjects by IV consisted of a feeling of warmth in the upper body that gradually became localized circumorally, a feeling of warmth in the throat that according to three of nine complainants was associated with a taste similar to that of menthol, numbness in and around the mouth and a definite hypoalgesia of that area in response to pricking with a pin, and a feeling that the eyeballs had enlarged and become heavy. Most of these symptoms subsided within 1-2 h.

In a later report,[79,168] Sidell and Groff told of giving a 25% aqueous solution of IV to 13 men by mouth, using doses of 1, 3, 5, 7, and 9 g. The smallest dose produced a plasma concentration of the oxime so low that measurements of it were of questionable significance; the results from that dose were not analyzed further. The peak plasma concentrations produced by the doses of 3, 5, 7, and 9 g were 1.9, 3.19, 4.41, and 5.56 µg/ml, respectively. Both I and II produced larger plasma concentrations with a given dose than IV; the ratio between the plasma concentrations produced by a given dose of II and the same dose of IV varied between 1.44 and 2.0, and the same ratio for I and IV varied between 1.44 and 2.6. The half-time for removal of IV from plasma was about 159 min, and that for urinary excretion of IV was about 288 min. The latter time is not only considerably longer than the corresponding times for I and II, but also much longer than that for removal from the plasma. The last discrepancy may indicate metabolic breakdown of the compound in the kidney. Karyotypic study of three of the subjects found no unusual number of gaps and breaks or increased mitotic index.

Sidell *et al.*[169] compared I, II, and IV with respect to absorption, distribution, and excretion in human subjects. The oximes were injected intravenously, I and II at 5 mg/kg and IV at 0.5 and 1.0 mg/kg. The lines for the plasma concentration of oxime at various times after the injections of I, II, and the larger dose of IV were nearly identical. The smaller dose of IV yielded plasma concentrations 0.5-5.0 µg/ml below those produced by the larger dose. The renal clearances of I and II were 606 and 644 ml/min, respectively, whereas that of IV was 95 ml/min. Although no independent measure of glomerular filtration was reported, the last value for renal clearance is well below the generally accepted mean for glomerular filtration in the human kidney of 126 ml/min and could be smaller than glomerular filtration in the particular subjects used in the study. If the clearance of IV really is smaller than the glomerular filtration, that would mean that the kidneys have a tubular reabsorption mechani[illegible] IV, whereas the tubular epithelium secretes I and II into the tubular lumina. The volumes of distribution of I and II were 815 and 775 ml/kg, respectively, whereas that of IV was only 173 ml/kg. The central compartment accounted for 33.2% and 25.3% of the total volumes of distribution of I and II, respectively, and for 58.0% of that for IV. The half-times for removal of oxime from the plasma were 79 min for I, 85 min for II, and 72 min for IV. Half-times for urinary excretion of the oximes were not given, but values for I and II can be estimated from curves in the report of the cumulative percentages of the doses excreted in the urine during various times. The half-times for urinary excretion estimated from these curves were 39 min for I and 48 min for II. After intramuscular injection of IV, the half-time for its urinary excretion was about 85 minutes;[77] the half-time for excretion after intravenous injection might be smaller by about 20 min, the minimal time required for reaching the peak plasma concentration of the oxime after intramuscular injection. The half-time for urinary excretion of IV may be about 1 h after intravenous injection.

GENERAL COMMENT

None of the oximes used in experiments with human subjects by the personnel of the Biomedical Laboratory at Edgewood Arsenal or of its contractors has given evidence of being dangerously toxic in the single doses used. The studies detailed in the body of this review have shown that the lethalities of the various oximes are not due solely to the content of the oximate radical in these compounds. The bispyridinium bisoximes (III and IV) seem to be more curaremimetic and more disturbing of normal gastrointestinal function than the monopyridinium monoximes (2-PAM I, I, and II) and the ketoxime (V). The most marked effect of both the monopyridinium monoximes and the bispyridinium bisoximes on functions in subjects poisoned by OP compounds is on neuromuscular transmission. The bispyridinium bisoximes are absorbed from the gastrointestinal tract less rapidly than the monopyridinium monoximes, but are more efficient in yielding a comparatively high plasma concentration of oxime than the monopyridinium monoximes, by virtue of having smaller volumes of distribution within the body and being excreted less rapidly by the kidneys.

The oximes, although they are metabolized to some extent, are removed from the body largely by excretory processes. Particularly for the monopyridinium monoximes, the kidneys are important excretory organs. Renal insufficiency has been found to increase the toxicities of these oximes significantly, but to have no definite effect on that of the ketoxime.

Most of these compounds are removed from the human body so rapidly that there is comparatively little hazard of cumulative toxicity from administration of reasonable doses of the oximes. The principal exception is V, which has been found to act synergistically with depressant barbiturates[170] and to induce significant changes in CNS function when repetitive doses were administered. The discovery that IV also appears in the CSF comparatively rapidly after its administration suggests that its potential for exerting toxic effects on CNS function during a course of repetitive administrations should be evaluated carefully. III also has been found to produce bothersome, but apparently not particularly dangerous, effects after repetitive doses.

Whereas the most striking cardiovascular response to the monopyridinium monoximes is hypertension, that to the bispyridinium bisoximes is prolonged hypotension after an initial, short hypertensive response. The hypertension and tachycardia that have been induced by initial doses of oximes have been found not to persist during a series of repetitive administrations. Furthermore, sympatholytic compounds have been found to be effective antagonists of these actions that could be used to control bothersome cardiovascular symptoms after the use of oximes.

The principal lack in the available information perceived in this review is the absence of any significant attempt to determine whether a delayed toxic effect becomes evident after administration of these compounds. Although hydrogen cyanide is a metabolic product of the monopyridinium monoximes and a nitrile has been found in the urine of rats given III, the production of these compounds has not been great enough to cause obvious toxic effects near the times of administration of the oximes. There is a slight possibility that repetitive administrations of oximes during sufficient periods could induce damage to crucial organs by continued production of low concentrations of hydrogen cyanide and nitriles in the body. Studies of the toxicities of these compounds during long-term administrations should be designed to afford information not only on chronic toxicity itself, but also on the possibility that oximes may have some degree of carcinogenic or mutagenic potenital. Their teratogenic potential should also be evaluated, although the existing information[171] suggests that there will be no effect of that sort.

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